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Regular Articles

L-Arabinose induces D-galactose catabolism via the Leloir pathway in *Aspergillus nidulans*

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ABSTRACT

L-Arabinose and D-galactose are the principal constituents of L-arabinogalactan, and also co-occur in other hemicelluloses and pectins. In this work we hypothesized that similar to the induction of relevant glycoside hydrolases by monomers liberated from these plant heteropolymers, their respective catabolisms in saprophytic and phytopathogenic fungi may respond to the presence of the other sugar to promote synergistic use of the complex growth substrate. We showed that these two sugars are indeed consumed simultaneously by *Aspergillus nidulans*, while L-arabinose is utilised faster in the presence than in the absence of D-galactose. Furthermore, the first two genes of the Leloir pathway for D-galactose catabolism – encoding D-galactose 1-epimerase and galactokinase – are induced more rapidly by L-arabinose than by D-galactose eventhough deletion mutants thereof grow as well as a wild type strain on the pentose. D-Galactose 1-epimerase is hyperinduced by L-arabinose, D-xylose and L-arabitol but not by xylitol. The results suggest that in *A. nidulans*, L-arabinose and D-xylose – both requiring NADPH for their catabolisation – actively promote the enzyme infrastructure necessary to convert β-D-galactopyranose via the Leloir pathway with its α-anomer specific enzymes, into β-D-glucose-6-phosphate (the starting substrate of the oxidative part of the pentose phosphate pathway) even in the absence of D-galactose.

1. Introduction

Pezizomycotina is a subphylum of *Ascomycota*, which comprises many saprophytic and plant pathogenic fungi, feeding on living or dead plant material. Fungal degradation of (plant) polysaccharides involves complexes of hydrolytic enzymes that act in synergy to liberate all the constituent monosaccharides for internal turnover. Expression of quite a few of these hydrolytic enzymes (glycoside hydrolases) is induced by the monosaccharide(s) they (help) liberate although in most cases, the most potent induction occurs in the presence of the polymeric substrate, suggesting that the typical physiological inducers are liberated gradually and present in limiting amounts that do not provoke carbon catabolite repression.

L-Arabinogalactan occurs in two main configurations called type I- and type II arabinogalactan. The backbone contains principally β-linked D-galactopyranose, [1,4] in type I and [1,3]/[1,6] in type II, while the side chain decoration mainly consist of α-linked L-arabinofuranose units (Sakamoto and Ishimaru, 2013; Knoch et al., 2014). α-L-Arabinofuranosidase AbfB (EC 3.2.1.55) is implicated in the first phase of the extracellular degradation as it hydrolyses [1,5]-, [1,3]- and [1,2]-α-linkages in polymers containing terminal non-reducing L-arabinofuranose (Rombouts et al., 1988). In *Aspergillus niger*, this family 54 glycoside hydrolase (GH54) is induced on arabinogalactan but also by (monomeric) L-arabinose (van der Veen et al., 1991, 1993). More recently, it had been demonstrated that expression of the corresponding gene (*abfB*) is controlled by the L-arabinose responsive transcriptional activator AraR, which also regulates (intracellular) L-arabinose catabolism (Battaglia et al., 2011a,b). The backbone of type I arabinogalactan is degraded by endo-β-[1,4]-D-galactanase (EC 3.2.1.89), which (eventually) produces β-1,4-galactobiose and D-galactose (de Vries et al., 2002; Michalak et al., 2012). This GH53 gene is expressed on L-arabinose in an *A. niger* CreA mutant (de Vries et al., 2002). In *A. niger*, the dimer is extracellularly split by a GH35 β-D-galactosidase (EC 3.2.1.23) while in *A. nidulans*, galactobiose is taken up and split intracellularly by GH2 β-D-galactosidase BgaD.

The *A. nidulans* (*bgaD/lacpA*) gene cluster encoding the GH2 β-D-

Abbreviations: AMM2, modified Aspergillus Minimal Medium; CDS, coding sequence; G6P, glucose-6-phosphate; GH, glycoside hydrolase; ORP, oxidoreductive pathway; PCP, pentose catabolic pathway; PPP, pentose phosphate pathway

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Table 1
Aspergillus nidulans strains used in this study.

Strain	Genotype	Reference
R21[a] (ATCC 48756)	*pabaA1 yA2*	Fantes and Roberts (1973)
TN02A3 (FGSC A1149)	*pyroA4; pyrG89; ΔnkuA::argB*	Nayak et al. (2006)
LKEF_003	*ΔgalmB::Af.pyroA*[b]	Kulcsár et al. (2017)
AOEF_006	*pyrG89; ΔgalE::Af.pyroA*[b]*; ΔnkuA::argB*	This work
Glasgow G094[c]	*biA1*; *wA3*; *araA1*	Clutterbuck (1981); de Vries et al. (1994)

The references given refer to the mutations relevant to this work; other markers are in standard use (cf. Clutterbuck, 1993). We have verified the growth phenotypes of the strains, mutant in *galE*, *galmB* or *ladA* (i.e., *araA1* loss-of-function mutation) on D-galactose and L-arabinose on AMM2 plates (Fig. 4).

[a] Wild-type reference for D-galactose and L-arabinose utilisation.

[b] *A. fumigatus* pyridoxine biosynthesis (*pyroA*) gene.

[c] The *araA1* missense mutation in strain Glasgow G094 (GenBank MH794597) is located in the coding region of the L-arabitol dehydrogenase (*ladA*) gene (locus AN0942; Flipphi et al., 2009).

galactosidase and the lactose transporter has previously been studied (Fekete et al., 2012). The expression of the genes comprised in this cluster is co-induced at the transcript level by D-galactose as well as by L-arabinose. A possible explanation is that the stereochemical configuration of the alcohol groups of the carbon atoms of the hemiacetal- or hemiketal rings is identical in D-galactose and L-arabinose and that in solution, these sugars thus strongly resemble each other in structure. However, the induction of *bgaD* and *lacpA* by L-arabinose may also suggest that this pentose signals the presence of L-arabinose-containing heteropolysaccharides, like L-arabinogalactan, in the medium and that L-arabinose may cross induce the Leloir pathway of D-galactose catabolism for (the imminent) simultaneous consumption of both sugars.

In this work, we investigated whether L-arabinose and D-galactose actively facilitate catabolism of one another in the genetic model fungus, *A. nidulans*.

2. Materials and methods

2.1. Strains and cultivation conditions

A. nidulans strains used in this study are listed in Table 1. *Aspergillus* Minimal Medium (AMM2) for shake-flask cultures was described by Fekete et al. (2002); carbon sources were used at 1% (w/v) initial concentration (and thus were present in equal mass amounts, not at equimolar amounts). Strains were supplemented for their respective auxotrophies. Liquid cultures were inoculated with 10^6 *A. nidulans* conidia (ml medium)$^{-1}$, collected from a fresh spore plate with matured vegetative spores. Shake-flask cultures were incubated at 37 °C in 500-ml Erlenmeyer flasks containing 100 ml medium in a rotary shaker (Infors HT Multitron, Infors AG, Bottmingen, Switzerland) at 200 rpm. Agar-solidified medium was used for conventional growth testing. Plates were “point inoculated” with a single drop of a freshly prepared suspension of vegetative spores at low density and incubated at 37 °C.

For transcript analyses, replacement cultures were used for which mycelia were pregrown for 24 h in AMM2 containing 1% (v/v) glycerol, and harvested by filtration. After thoroughly washing, mycelia were transferred to flasks with carbon-free AMM2 and then incubated for 1 h at 200 rpm, before the monosaccharides to be tested were added to a concentration of 1% (w/v) (i.e., 10 g L^{-1}). Samples were taken after 8 and 24 h of continued submerged incubation.

2.2. Generation and verification of galE deletion strains

The primers and plasmids used in Δ*galE* mutant strain generation are listed in Table S1. A gene deletion cassette was constructed *in vitro* with the double-joint PCR method (cf. Yu et al., 2004). The *galE* deletion cassette contained the *A. fumigatus pyroA* gene (Nayak et al., 2006) which served as primary selection marker. Protoplasts of *A. nidulans* uridine- and pyridoxine-auxotrophic strain TN02A3 (cf. Nayak et al., 2006) were transformed with 10 µg of the linear deletion construct. This transformation host facilitates the acquisition of knockouts by homologous recombination at the *galE* locus due to the absence of a functional non-homologous end-joining machinery (Δ*nkuA*::*argB*, see Table 1). Pyridoxine-prototrophic transformants were probed for the absence of *galE* coding sequences by PCR, primed off genomic DNA using gene-specific primers. Transformants from which the *galE* gene was absent were purified twice to single cell colonies and maintained on selective minimal medium plates. One of the *galE* deletion strains thus obtained was chosen for use in this work after verifying the expected, severe D-galactose growth phenotype on plates. The deletion phenotype was found reverted to wild type growth upon complementation of the chosen Δ*galE* strain with the functional *galE* gene integrated at an ectopic locus (results not shown).

2.3. Genomic DNA and total RNA isolation and Northern blot analysis

Samples of *A. nidulans* mycelia were collected and processed as described by Fekete et al. (2012). For nucleic acid isolation, frozen biomass was ground to dry powder under liquid nitrogen, using chilled mortar and pestle. Genomic DNA and total RNA were extracted using Macherey-Nagel’s (Düren, Germany) NucleoSpin Plant II and NucleoSpin RNA Plant isolation kit, respectively, according to the manufacturer’s protocol. Northern analyses were conducted as described previously (Fekete et al., 2016). The oligonucleotide primers used to PCR digoxigenin-labelled probes (Roche Applied Science, Penzberg, Germany) are given in Supplementary Table S1; Genomic DNA from R21 was used as the template.

2.4. Determination of residual sugar concentrations

Residual D-galactose and L-arabinose concentrations in the growth medium were determined by High Performance Liquid Chromatography (HPLC; Gilson Inc., Middletown, WI, USA) with refractive index detection, as described by Fekete et al. (2002). Sugar utilisation rates were calculated from the highest decrease in residual concentrations between two subsequent samplings.

2.5. Reproducibility

The kinetic data presented, i.e., profiles for the residual sugar concentration in the medium with time, are the means of three independent fermentations (biological triplicates). Data were analysed and visualised with SigmaPlot (Jandel Scientific, San Jose, CA, USA), and standard deviations (SDs) were determined. Transcript analyses were performed independently with RNAs isolated from at least three replicate cultures.

3. Results

3.1. Aspergillus nidulans simultaneously consumes D-galactose and L-arabinose

On D-glucose and L-rhamnose as a mixed carbon source, the latter sugar was not taken up by *A. nidulans* before the “preferred” D-glucose is exhausted, regardless whether the strain was a wild type or a carbon derepressed *creA* mutant (Tamayo-Ramos et al., 2012). The consumption of L-rhamnose was thus inhibited at the level of uptake not involving the DNA-binding carbon catabolite repressor CreA. We therefore first assessed whether or not *A. nidulans* utilises D-galactose and L-arabinose simultaneously. Fig. 1 shows that the fungus takes up and

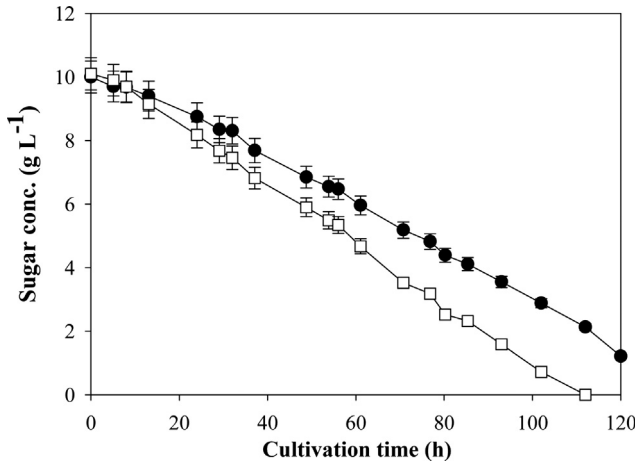

Fig. 1. Time profile of residual D-galactose (filled circles) [●] and L-arabinose (open squares) [□] concentration in batch cultures of *A. nidulans* R21 (wild-type) strain in minimal medium initially containing 10 g L^{-1} D-galactose and 10 g L^{-1} L-arabinose. Media were inoculated with conidia collected from a fresh spore plate with matured vegetative spores. Standard deviations are given with bars for each determined concentration; for the last determinations for L-arabinose, the bar is smaller than the symbol that marks the time point. For further details, see Section 2.

consumes these two sugars concomitantly, the opposite of the situation with D-glucose and L-rhamnose. The performance of the two sugars appears similar when cultured individually, as both are exhausted by 72 h of cultivation, starting from 10 g L^{-1} (see below, Section 3.3). In the mixed culture (10 + 10 g L^{-1}), L-arabinose was consumed faster than D-galactose on mass base. We could thus avoid to employ more elaborate cultivation methods such as chemostat cultures at low dilution rates and limited carbon flux to circumvent carbon catabolite repression (Ilyés et al., 2004), and considered batch cultures suitable to investigate synergistic effects during concurrent L-arabinose and D-galactose catabolism.

3.2. Selection of diagnostic genes of D-galactose- and L-arabinose catabolism

To monitor the transcription of structural genes involved in catabolism of L-arabinose and D-galactose in response to either of these two sugars, we selected those genes whose involvement was proven with loss-of-function mutants in *A. nidulans*.

The ubiquitous pathway for the utilisation of D-galactose is the Leloir pathway (Fig. 2) in which D-galactose is essentially epimerised to D-glucose, and the first dedicated step is the phosphorylation of α-D-galactopyranose by galactokinase (GalE: Locus AN4957) (Roberts, 1970; Alam and Kaminskyj, 2013; Orosz et al., 2014). Galactokinase (EC 2.7.1.6) is specific in its use of the α-anomer of D-galactopyranose (Howard and Heinrich, 1965). Recently, we have shown that an intracellular galactose-1-epimerase (GalmB: Locus AN3432; EC 5.1.3.3), which interconverts the α- and β-anomers of D-galactose in solution, contributes to the flux through the Leloir pathway (Kulcsár et al., 2017). The last three enzymes of the Leloir pathway have important anabolic functions in the production of cell wall precursors UDP-glucose and UDP-galactose (El-Ganiny et al., 2010). Consequently, they are expressed constitutively and thus transcription of the encoding genes *ugeA*, *galF*, and *pgmB* was not considered in this study.

L-Arabinose is catabolised through an oxidoreductive pathway (pentose catabolic pathway: PCP; Fig. 2) in which the reduction reactions are catalysed by NADPH-dependent oxidoreductases and the oxidations are performed by NAD^+-dependent oxidoreductases (Chiang and Knight, 1961; Witteveen et al., 1989; de Vries et al., 1994). The equilibria in the pathway lead to accumulation of the polyol intermediates. Catabolism is driven by the phosphorylation of D-xylulose by the highly specific xylulokinase (XkiA: Locus AN8790; EC 2.7.1.17) (VanKuyk et al, 2001; Bunker et al., 2013). All enzymes of the PCP act on the linear form of their sugar substrates and interconversion of anomers would thus appear irrelevant for the flux through L-arabinose catabolism. Indeed, deletion of *galmB* did not affect the growth on L-arabinose by *A. nidulans* (see Fig. 3B).

A classical *A. nidulans* mutant called *araA1* that cannot grow on L-arabinose and L-arabitol on plates was shown to lack L-arabitol:NAD^+ dehydrogenase- as well as L-xylulose:NADH reductase activity (EC 1.1.1.12) (de Vries et al., 1994). Interestingly, the L-arabitol dehydrogenase mutant *araA1* could also not grow on galactitol, the product

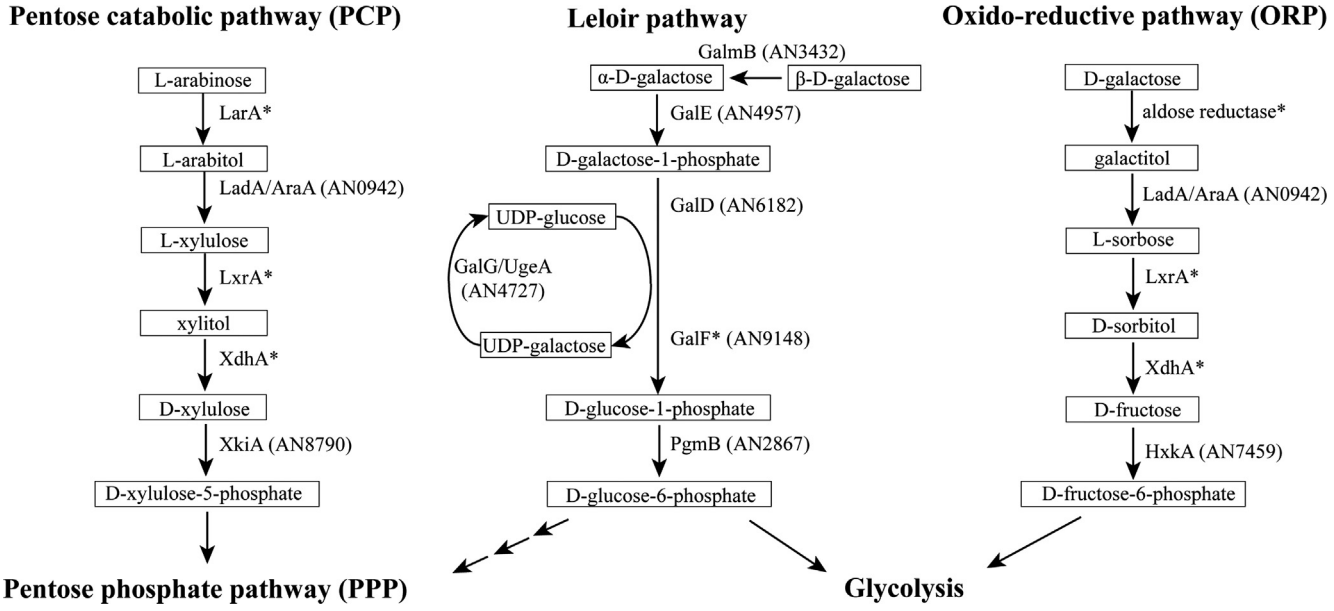

Fig. 2. Schematic representations of the pentose catabolic pathway (PCP) for L-arabinose (left), the Leloir pathway (centre) and the minor alternative oxidoreductive pathway (ORP) for D-galactose catabolism (right) in *Aspergillus nidulans*. The enzymes that catalyse particular conversions and the corresponding locus numbers in the *A. nidulans* annotated genome are indicated (Flipphi et al., 2009; Kowalczyk et al., 2015). Unidentified or unconfirmed enzymes (genes/loci) in *A. nidulans* are marked with a star [*].

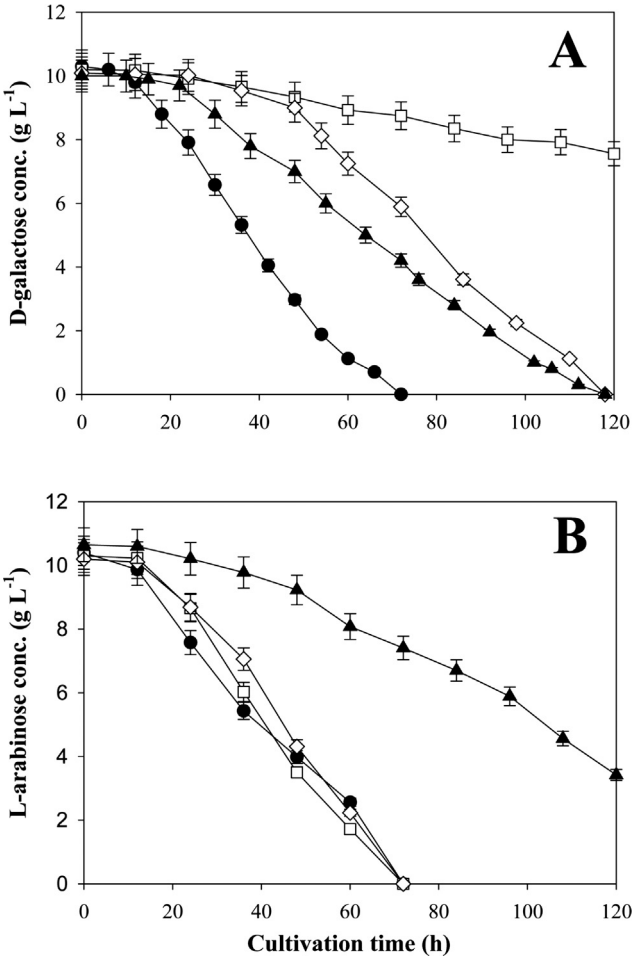

Fig. 3. Panel A. Time profile of residual carbon source concentration of *A. nidulans* wild type and mutant strains in AMM2 initially containing 10 g L^{-1} D-galactose. Panel B. Time profile of residual carbon source concentration of *A. nidulans* wild type and mutant strains in AMM2 initially containing 10 g L^{-1} L-arabinose. The reference strain (R21) is indicated by circles [●], the *galE* deletant by squares [□], the *araA1* loss-of-function mutant strain by triangles [▲], and the *galmB* deletant by diamonds [◇]. Media were inoculated with conidiospores collected from a fresh spore plate with matured vegetative spores. For further details, see the Legend to Fig. 1 and Section 2.

of aldose reductase activity on D-galactose. Fekete et al. (2004) demonstrated that aldose reductase- and L-arabitol dehydrogenase activities catalyse the first two steps of an alternative, oxidoreductive pathway for the utilisation of D-galactose in *A. nidulans* (ORP) (Fig. 2). The past investigations thus strongly suggest that alternative catabolism of D-galactose (ORP) in *A. nidulans* shares (at least) one enzyme – L-arabitol dehydrogenase (LadA: Locus AN0942) – with L-arabinose utilisation (PCP). However, beyond L-arabitol dehydrogenase, the genetic components of the ORP have not been unambiguously identified in *A. nidulans* to date.

3.3. Phenotype of the selected mutants on D-galactose and L-arabinose

Fig. 3 shows the effects of two gene deletions in galactokinase (Δ*galE*) and galactose-1-epimerase (Δ*galmB*), respectively, and the loss-of-function mutation in L-arabitol dehydrogenase (*araA1*) on the consumption of D-galactose and L-arabinose in liquid cultures. Fig. 4 shows growth of these strains on plates at two different time-points. All three mutations affect the consumption of D-galactose, Δ*galE* having by far the biggest impact (Fig. 3A and Table 2). Residual growth of the *galE* deletant strain by means of the alternative pathway (OPR) was observed

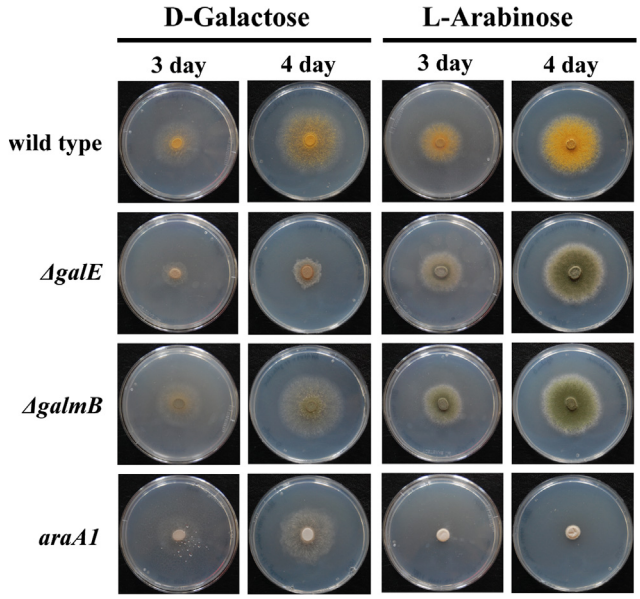

Fig. 4. Point-inoculated ("single colony") plate cultures of *A. nidulans* strains grown on agar-solidified minimal medium with 10 g L^{-1} D-galactose or L-arabinose as the sole source of carbon. Plates were "point inoculated" with a single drop of a freshly prepared suspension of vegetative spores at low density and incubated at 37 °C. The colonies were inspected daily and documented at 72 and 96 h after inoculation. For further information regarding the mutant strains, see Clutterbuck (1981), de Vries et al (1994), Fekete et al (2004) and Kulcsár et al (2017).

Table 2
Sole carbon consumption rates in *A. nidulans* mutant strains relative to the wild type reference R21 (mean of three biological replications in Fig. 3).

Strain	Carbon source	Consumption rate (g L^{-1} h^{-1})	Relative consumption rate (%)
R21 (wild type)	D-galactose	0.19	100
	L-arabinose	0.16	100
Δ*galE*	D-galactose	0.02	12.63
	L-arabinose	0.18	113.46
Δ*galmB*	D-galactose	0.12	64.73
	L-arabinose	0.17	110.25
araA1 mutant	D-galactose	0.11	55.78
	L-arabinose	0.07	44.87

(Fig. 4), in accordance with Fekete et al. (2004). Indeed, lack of the ORP enzyme L-arabitol (galactitol):NAD^+ dehydrogenase (*araA1* mutant) slowed down D-galactose utilisation such that it took five instead of three days before it was exhausted. The performance on D-galactose of the strain lacking mutarotase (Δ*galmB*) is more similar to that of the G094 mutant (Fig. 3A). On the other hand, the galactokinase and galactose mutarotase deletions do not appear to have profound effects on the utilisation of L-arabinose (Fig. 3B and Table 2). The L-arabitol dehydrogenase mutant (*araA1*) is clearly delayed; ~75% of the carbon source still available by the time the L-arabinose is exhausted in the wild type, the galactokinase- and the 1-epimerase loss-of-function mutants.

3.4. Transcript analysis of the galmB, galE, ladA and xkiA genes

We probed the transcription of the three genes functionally involved in D-galactose catabolism in parallel with that of the D-xylulokinase (*xkiA*) gene, which is only involved in pentose catabolism, during batch cultivation of the wild type strain. A representative Northern analysis is shown in Fig. 5. As expected, expression of *ladA* is induced by both sugars as it is implicated in the catabolisms of either (de Vries et al.,

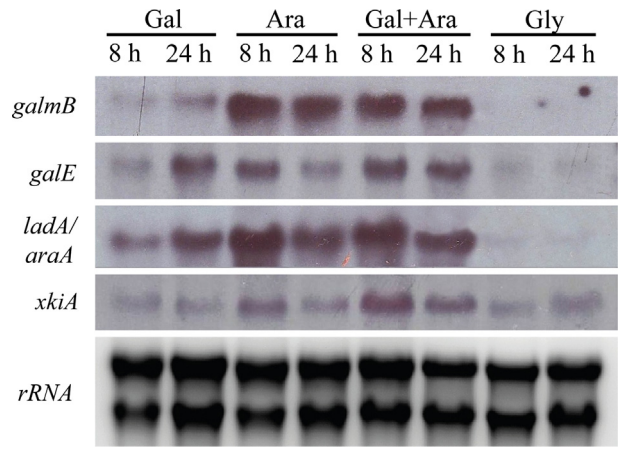

Fig. 5. Transcript analysis of the *A. nidulans galmB, galE, ladA* and *xkiA* genes in the wild-type strain R21 in response to D-galactose (Gal), L-arabinose (Ara) and D-galactose plus L-arabinose (Gal + Ara). Glycerol (Gly) serves as a neutral carbon source, i.e., neither inducing nor repressing these four genes. rRNA (28S and 18S) was visualized with ethidium bromide and is shown as a control for RNA sample quality and quantity.

1994; Fekete et al., 2004), albeit in the L-arabinose cultures it is expressed to higher levels and earlier after inoculation. The ORP is the minor alternative of the Leloir pathway for the catabolism of D-galactose (Fekete et al., 2004; Christensen et al., 2011; Orosz et al., 2014; Kowalczyk et al., 2015). *xkiA* has a high basal level of expression and some overexpression is only apparent on L-arabinose at 8 h after mycelial transfer. In contrast, *galE* appears to respond to both sugars, again seemingly earlier in the L-arabinose cultures. Interestingly, the Northern analysis suggests that the overall response to L-arabinose is of the same order as that to D-galactose. This is remarkable because GalE is not involved in L-arabinose catabolism as it catalyses the phosphorylation of α-D-galactopyranoside (Leloir pathway). Furthermore, the response of the *galmB* to L-arabinose appears to be one order of a magnitude higher than the induction observed in the presence of D-galactose, the anomers of which are the principal substrates of the intracellular mutarotase. *galmB* does respond to D-galactose as its transcript is hardly visible in the control samples (glycerol). This hyperinduction of *galmB* on L-arabinose seems physiologically aberrant, as deletion of the mutarotase gene does not lead to a growth phenotype on this pentose (Fig. 4). Indeed, all enzymes of the PCP are exclusively active on the open form of the aldose and ketose intermediates, and not on their anomeric hemiacetal or hemiketal forms.

On the mixed carbon source of L-arabinose and D-galactose, the four diagnostic genes essentially behaved like they did in response to L-arabinose alone at 8 h (Fig. 5). At 24 h, the *galmB* and *ladA* induction levels remained similar to their respective responses in the L-arabinose-alone cultures, clearly better expressed than on D-galactose alone. Conversely, *galE* is now expressed to higher levels on the mixed carbon source than on L-arabinose alone. Both sugars are still abundantly present in the medium at 24 h. However, from our results we cannot conclude whether the galactokinase gene is responding exclusively to D-galactose at 24 h or whether there is (still) an additive effect from the co-presence of L-arabinose.

4. Discussion

Of the two principal monosaccharide units of L-arabinogalactan, the (main) sugar constituting the side chains, L-arabinose, is forcibly liberated before the (main) sugar constituting the backbone, D-galactose. Consistent in our analysis is that the genes encoding the first two enzymes in the catabolic Leloir pathway are both responding to the presence of L-arabinose, as if this pentose signals the (imminent) co-presence of D-galactose. This is collaborated by the fact that the responses to L-arabinose are faster and more outspoken (at 8 h) than those to D-galactose, including on the mixed carbon source. Our experiment thus strongly suggests that L-arabinose effectively cross induces catabolism of D-galactose via the Leloir pathway to facilitate their simultaneous uptake and catabolism, in a similar way as it induces glycoside hydrolases like endo-1,4-β-D-galactanase (*galA* in *A. niger*; de Vries et al., 2002) and β-1,4-D-galactosidase (*bgaD* in *A. nidulans*; Fekete et al., 2012) involved in the degradation of the β-galactan backbone of arabinogalactans.

At first sight, there appears to be a contradiction between the cross induction of the Leloir pathway and the hyperinduction of *galmB* by L-arabinose, and the fact that D-galactose is less rapidly consumed in the mixed culture than when present as sole carbon source. We speculate that the full capacity of the oxidoreductive paths (L-arabitol dehydrogenase in particular) is employed for the catabolism of L-arabinose in the mixed carbon source, while the concurrent D-galactose utilisation proceeds exclusively via the Leloir pathway, expedited by L-arabinose cross induction. In other words, D-galactose catabolism in the mixed cultures completely misses the contribution of the ORP via galactitol. L-Arabitol accumulates during L-arabinose utilisation (de Vries et al., 1994) to compete with galactitol in the mixed cultures. Noteworthy, two paralogue L-arabitol dehydrogenases in *A. niger* called LadA (orthologue of the product of the *A. nidulans* oxidoreductase gene at locus AN0942) and LadB (orthologue of the product of the gene at AN4336), have higher affinity for L-arabitol than for galactitol (Mojzita et al., 2012).

In contrast, the utilisation of L-arabinose in the mixed cultures benefits from the concurrent catabolism of D-galactose via the Leloir pathway. The major building blocks of the fungal cell wall, UDP-glucose and UDP-galactose, are normally synthesized from the key metabolic intermediate glucose-6-phosphate (G6P) via the Leloir pathway for D-galactose in the anabolic direction (El-Ganiny et al., 2010). Some *A. nidulans* mutants deficient for phosphoglucomutase activity (EC 5.4.2.2) do not grow on any source of carbon, unless supplemented with D-galactose (Boschloo and Roberts, 1979). Additionally, on non-glycolytic substrates, radiolabeled galactose ends up primarily in the fungal cell wall (Katz and Rosenberger, 1970). Hence, in a mixed carbon source of L-arabinose and D-galactose, no carbon flux from L-arabinose is required to synthesize the cell wall precursors.

A second advantage from the presence of the D-galactose co-substrate could be that it provides for an alternative source of G6P, the starting metabolite of the oxidative part of the pentose phosphate

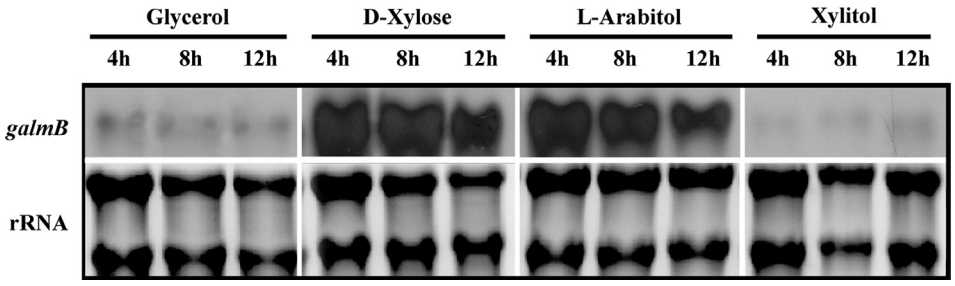

Fig. 6. Transcript analysis of *galmB* gene expression in response to D-xylose, L-arabitol and xylitol in *A. nidulans* strain R21. Glycerol serves as a neutral carbon source, i.e., neither inducing nor repressing this gene. rRNA (28S and 18S) was visualized with ethidium bromide and is shown as a control for RNA sample quality and quantity.

pathway (PPP), which generates NADPH (Chen et al., 2015; Wasylenko et al., 2015). L-Arabinose conversion to D-xylulose requires two NADPH and the oxidation of G6P to ribulose-5-phosphate yields two NADPH. When L-arabinose is the sole carbon source, G6P is synthesized gluconeogenically from fructose-6-phosphate formed by the non-oxidative part of the PPP from D-xylulose-5-phosphate (the endproduct of the PCP). Consequently, the carbon flux needs to pass the non-oxidative part of the PPP twice before becoming available for further metabolisation. The (direct) provision of G6P from D-galactose via the Leloir pathway can make this metabolic loop less important for the recycling of all NADPH, *de facto* accelerating the utilisation of L-arabinose. To test this thesis, we probed the expression of *galmB* in the wild type *A. nidulans* strain after mycelial transfer to three other carbon sources catabolised via PCP, i.e., L-arabitol (requires 1 NADPH), D-xylose (idem) and xylitol (no NADPH required) (Fig. 2). As shown in Fig. 6, we found that *galmB* was indeed clearly overexpressed upon mycelial transfer to medium containing D-xylose or L-arabitol, similar to its response to L-arabinose (Fig. 5), but not upon transfer to xylitol. Our results thus suggest that in *A. nidulans*, at least certain carbon sources that require NADPH for their catabolisation actively promote the enzyme infrastructure to take up and convert D-galactose to G6P via UDP-galactose, even in the absence of D-galactose.

The hyperinduction of the intracellular D-galactose 1-epimerase by L-arabinose is consistent with the channeling of the flux from D-galactose through the Leloir pathway towards the PPP. Both galactokinase and phosphoglucomutase are α-anomer-specific enzymes (Howard and Heinrich, 1965; Salas et al., 1965; Wurster and Hess, 1974). Conversely, the first enzyme of oxidative part of the PPP, G6P dehydrogenase (EC 1.1.1.49), only uses β-G6P as its substrate (Smith and Beutler, 1966). In fungi, plants and bacteria, the interconversion of the anomeric hemiacetal forms of G6P is catalysed by a dedicated G6P 1-epimerase (EC 5.1.3.15), inert towards non-phosphorylated sugars (Wurster and Hess, 1972; 1973; Graille et al., 2006). In *A. nidulans*, it is encoded at locus AN10222. A dedicated 1-epimerase is absent in mammalians where G6P anomerisation is performed by an “ordinary” mutarotase, i.e., the orthologue of GalmB (EC 5.1.3.3) (Gernert and Keston, 1974). We contemplate the possibility that GalmB is accessory to G6P 1-epimerase in funneling the D-galactose carbon flux into PPP to recycle NADPH used in L-arabinose catabolisation.

Furthermore, all but one fungal glycoside hydrolases involved in extra- or intracellular breakdown of β-galactans are of the retaining type, i.e., the stereochemical configuration at C_1 remains “β” after hydrolysis of the bond between the D-galactose units; these enzymes belong to the families GH1, GH2, GH5, GH16, GH30, GH35, or GH53 (The CAZypedia Consortium, 2018). The exception is exo-β-1,3-D-galactanase (EC 3.2.1.145), a GH43 enzyme (*A. nidulans* locus AN7313) involved in the breakdown of type-II arabinogalactan (cf. Ichinose et al., 2005). The monomeric end product of the fungal breakdown of the β-galactan moieties of arabinogalactans is thus β-D-galactopyranose rather than D-galactose. In the yeast *Kluyveromyces lactis*, uptake of D-galactose is β-anomer specific and strains deficient in mutarotase activity do not grow on D-galactose (Fukasawa et al., 2009, 2012). This fungus does not possess an alternative oxidoreductive pathway for D-galactose utilisation and the spontaneous interconversion of the anomers in the cytosol is apparently not sufficiently robust to sustain growth. In analogy, we hypothesize that in *A. nidulans*, mutarotase GalmB is key to the rapid intracellular conversion of β- into α-galactopyranose, to channel the D-galactose flux through the Leloir- and the PPP. Meanwhile, L-arabinose monopolizes the oxidoreductive path, particularly at the limiting concentrations of monosaccharides liberated during the breakdown of plant arabinogalactans.

5. Concluding remarks

In the post-omics age, science has rediscovered metabolism and physiology. Most of what is known about anomer specificity and anomer interconversion stems from biochemical and chemical work in the sixties, seventies and eighties of the last century. Our paper illustrates some of the challenges and opportunities provided by sugar anomers and anomer-specific enzymes to optimise L-arabinose catabolism in a saprophytic filamentous fungus of the *Aspergillus* genus by co-utilising D-galactose, the two principal constituents of the common hemicellulose arabinogalactan.

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Author contributions

EF, LKa, MVAP and RPdV conceived the study; EF, LKa and MF designed experiments; ZN, LKu and AO performed experiments; EF, MF, LKa, ZN, MVA-P and RPdV analysed data; LKa and RPdV obtained funding; MF, LKa, RPdV and EF wrote the manuscript; all authors read and approved the submitted manuscript.

Ethical statement

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fgb.2018.11.004.

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