FEMS Microbiology Letters, 364, 2017, fnx202

doi: 10.1093/femsle/fnx202
Advance Access Publication Date: 25 September 2017
Research Letter

RESEARCH LETTER – Physiology & Biochemistry

Identification of a mutarotase gene involved in D-galactose utilization in *Aspergillus nidulans*

László Kulcsár[1], Michel Flipphi[1], Ágota Jónás[1], Erzsébet Sándor[2], Erzsébet Fekete[1] and Levente Karaffa[1,*]

[1]Department of Biochemical Engineering, Faculty of Science and Technology, University of Debrecen, H-4032, Egyetem tér 1., Debrecen, Hungary and [2]Institute of Food Science, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, H-4032, Böszörményi út 138., Debrecen, Hungary

*Corresponding author: Department of Biochemical Engineering, Faculty of Science and Technology, University of Debrecen, H-4032, Egyetem tér 1., Debrecen, Hungary. Tel: +36 52 512 900 (ext. 62488); E-mail: karaffa.levente@science.unideb.hu

One sentence summary: A gene coding for mutarotase (aldose 1-epimerase) activity has been identified in the model fungus *Aspergillus nidulans* and is physiologically relevant for D-galactose utilization.

Editor: Matthias Brock

ABSTRACT

Aldose 1-epimerases or mutarotases (EC 5.1.3.3) are catalyzing the interconversion of α- and β-anomers of hemiacetals of aldose sugars such as D-glucose and D-galactose, and are presumed to play an auxiliary role in carbohydrate metabolism as mutarotation occurs spontaneously in watery solutions. The first step in the Leloir pathway of D-galactose breakdown is preceded by accelerated conversion of β-D-galactopyranose into the α-anomer, the substrate of the anomer-specific D-galactose 1-kinase. Here, we identified two putative aldose-1-epimerase genes (*galmA* and *galmB*) in the model organism *Aspergillus nidulans*, and characterized them upon generation of single- and double deletion mutant strains, as well as overexpressing mutants carrying multiple copies of either. Assaying cell-free extracts from the *galmB* single- and *galm* double mutants, we observed that the mutarotation hardly exceeded spontaneous anomer conversion, while *galmB* multicopy strains displayed higher activities than the wild type, increasing with the copy number. When grown on D-galactose in submerged cultures, biomass formation and D-galactose uptake rates in mutants lacking *galmB* were considerably reduced. None such effects were observed studying *galmA* deletion mutants, which consistently behave like the wild type. We conclude that GalmB is the physiologically relevant mutarotase for the utilization of D-galactose in *A. nidulans*.

Keywords: *Aspergillus nidulans*; D-galactose; aldose 1-epimerase; mutarotation; Leloir pathway

INTRODUCTION

Mutarotation is a form of tautomerization which occurs spontaneously when the hydroxyl group at the anomeric position (C-1) of a hemiketal or hemiacetal carbohydrate in solution switches configuration between α- and β-anomers. The enzyme (or enzyme activity) that catalyzes this interconversion is called mutarotase (synonym: aldose 1-epimerase; EC 5.1.3.3).

The soil-born saprophyte *Aspergillus nidulans* is a model organism for filamentous fungi. D-Galactose is catabolized here via two alternative pathways (Fekete *et al.* 2004). The Leloir pathway—which is operative in most pro- and eukaryotes—begins with ATP-dependent phosphorylation of α-D-galactopyranose to D-galactose-1-phosphate by galactokinase (EC 2.7.1.6: ATP: D-galactose 1-phosphotransferase) as the first dedicated reaction (Frey 1996). Galactokinase is an

Received: 30 May 2017; **Accepted:** 22 September 2017

anomer-specific enzyme that only accepts α-D-galactopyranose as its substrate (recent review: Conway and Voglmeir 2016). In contrast, the alternative, oxidoreductive pathway is initiated by a reduction of the open (linear) form of D-galactose to galactitol by a NADPH-dependent aldose reductase (EC 1.1.1.21; Fekete *et al.* 2004). This pathway eventually yields fructose-6-phosphate or tagatose-1,6-bisphosphate in *A. nidulans* (Flipphi *et al.* 2009).

In certain bacterial taxa, the genes encoding the first three enzymes of the Leloir route and the D-galactose mutarotase are organized under common promoter control in operons (Bouffard, Rudd and Adhya 1994; Grossiord *et al.* 2003). A similar organization of functionally related structural genes under coordinated expression control exists in some fungal taxa (Slot and Rokas 2010); the first eukaryotic galactose utilization gene cluster was uncovered in *Saccharomyces cerevisiae*. Remarkably, the *GAL10* gene codes for a bifunctional protein with both UDP-galactose 4-epimerase activity (EC 5.1.3.2; the third enzyme in the catabolic Leloir path) and mutarotase activity (Scott and Timson 2007). While one can thus consider that D-galactose mutarotase contributes to the catabolic flux through the Leloir pathway by converting β-D-galactopyranose into the galactokinase substrate α-anomer, this particular aspect of D-galactose utilization has scarcely been studied in filamentous Ascomycete fungi (Pezizomycotina), even though enzyme-catalyzed mutarotation was first described in *Penicillium chrysogenum* (Keilin and Hartree 1952). In this paper, we identified two putative aldose-1-epimerase genes (*galmA* and *galmB*) in *A. nidulans* and show that one of the gene products is physiologically relevant for the utilization of D-galactose.

MATERIALS AND METHODS

Aspergillus nidulans strains, media and culture conditions

Aspergillus nidulans strains and transformants used in this study are listed in Table S1 (Supporting Information). Aspergillus Minimal Medium (AMM) for shake-flask cultures was described by Pontecorvo *et al.* (1953). Carbon sources were used at 1.5% (w/v) initial concentration. Cultures were inoculated with 10^6 *A. nidulans* conidia (ml medium)$^{-1}$. Shake-flask cultures were incubated at 37°C in 500 ml Erlenmeyer flasks containing 100 ml culture aliquots in a rotary shaker (Infors HT Multitron, Infors AG, Bottmingen, Switzerland) at 200 rpm.

For enzyme activity determinations, replacement cultures were used for which mycelia were pre-grown for 24 h in AMM containing glycerol, and harvested by filtration. After washing the biomass with cold sterile water, mycelia were transferred to flasks with carbon-free AMM and were incubated for 1 h in a rotary shaker at 200 rpm, after which carbon sources were added. Samples were taken after 8 h of further incubation—a period in which maximal activity levels were achieved, with a minimal variation in the biomass concentration. For transcript analysis, mycelia were pre-grown for 24 h in AMM containing glycerol and after thorough washing, transferred to fresh medium containing galactose.

All chemicals were of analytical grade, and except where noted otherwise, were purchased from Sigma-Aldrich Kft., Budapest, Hungary.

Classical genetic techniques and transformation

Conventional genetic techniques were employed (Clutterbuck 1974). Progeny of sexual crosses were tested for known auxotrophies using standard techniques. *Aspergillus nidulans* transformations were performed as described by Tilburn *et al.* (1983). Transformants were purified twice to single cell colonies and maintained on selective minimal medium plates.

Genomic DNA and total RNA isolation

Mycelia were harvested by filtration, frozen in liquid nitrogen and ground to powder. Genomic DNA was extracted using a NucleoSpin Plant II kit whereas total RNA was isolated with a NucleoSpin RNA Plant kit (both kits from Macherey-Nagel GmbH & Co. KG, Düren, Germany). The concentration and purity were determined with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

Generation of knockout mutant strains

A gene deletion cassette was constructed using the double-joint PCR method (Yu *et al.* 2004). The *galmA* deletion cassette contained the *pyr4* gene from *Trichoderma reesei* (Gruber *et al.* 1990), while in the *galmB* deletion cassette, the *A. fumigatus pyroA* gene (Nayak *et al.* 2006) was used as primary selection marker. The oligonucleotide primers used are listed in Table S2 (Supporting Information). Protoplasts of *A. nidulans* uridine- and pyridoxine-auxotrophic strain TN02A3 were transformed with 10 μg of the linear deletion cassette. This transformation host facilitates the acquisition of gene knockouts due to the absence of functional non-homologous end-joining machinery (Nayak *et al.* 2006). Pyridoxine- or uridine-prototrophic transformants were probed for the absence of *galmA* or *galmB* coding sequences by PCR, using gene-specific primers. For the creation of the double mutarotase deletion mutants (*galmA/galmB*), the same *galmA* and *galmB* replacement constructs (5 μg of each) as described above were co-introduced into TN02A3. Selected pyridoxine and uridine-prototrophic, double deletion mutant strains were verified by PCR and then crossed out to reacquire the wild type *nkuA* gene. Offspring of two independently isolated primary double mutarotase mutants were routinely tested.

Reintroduction of *galmA/galmB* into gene-deleted backgrounds

Characterized first generation deletion mutants of *galmA* or *galmB* were each crossed with strain RJMP155.55 to exchange auxotrophic markers and rid the *nkuA* deletion. Pyridoxine/uridine-prototrophic and riboflavin-auxotrophic offsprings were verified by PCR for the presence of *nkuA*. Specific primers were used to amplify *galmA* and *galmB* (Table S2, Supporting Information). 10 microgram of amplification product was co-transformed with 1 μg pTN2 (carrying the *A. fumigatus riboB* gene; Nayak *et al.* 2006) into the appropriate Δ*nkuA*-cured gene deleted strain. Among the riboflavin-prototrophic transformants, the presence of the reintroduced gene was probed by PCR. The *galm* copy number was estimated by Southern blot analysis, and selected strains that had acquired functional *galmA/galmB* in one or more copies were phenotypically characterized.

Southern and northern blot analysis

Standard procedures (Sambrook and Russell 2001) were used. Probes were digoxigenin-labeled using a PCR DIG Probe Synthesis Kit (Roche Applied Science, Penzperg, Germany; Table S2). Hybridization was visualized with Lumi-Film Chemiluminescent Detection Film (Roche Applied Science).

Preparation of cell-free extracts

Twenty milliliters of culture broth were withdrawn and suction-filtered, and the retained biomass was washed with 20 mM Tris hydrochloride, pH 7.5. The mycelia were then resuspended in 5 ml of the same buffer and homogenized in an ice-cooled Potter-Elvehjem glass homogenizer equipped with an in-tube Teflon pestle. The homogenate was centrifuged at 8500 × g (5 min, 4°C), and the supernatant was either immediately used to assay mutarotase activity, or intact mycelia were stored at –75°C until used for enzyme assays.

Analytical methods

DCW (dry cell weight) was determined from 5 ml culture aliquots. Biomass was harvested onto a pre-weighted glass wool filter by suction filtration and washed with cold tap water after which the filter was air-dried at 80°C. The medium concentration of D-glucose and D-galactose was determined by HPLC (Gilson, Inc., Middleton, WI, USA) with refractive index detection as described by Fekete *et al.* (2002). Kinetic data presented are the means of three independent fermentations (biological replicates), where two samples (technical replicates) were taken from each culture at every time point. These two separate measurements never deviated by more than 14% for the DCW and by no more than 5% for the sugar concentration, respectively. The significance of changes in biomass- and residual sugar concentration in the growth medium of deletion mutants or complemented deletion strains, relative to the control cultures, was assessed using Student's t-test with probability (p) values given in the Results section.

The specific activity of mutarotase was measured by coupling the anomer epimerization of freshly solubilized α-D-glucose to an oxidation reaction catalyzed by the D-glucose dehydrogenase from a *Pseudomonas* sp. (EC. 1.1.1.47). This enzyme is specific for the β-anomer and uses NAD^+ (Bouffard, Rudd and Adhya 1994). The reaction was set up as described by Fekete *et al.* (2008). Initial epimerization rates in the absence of *A. nidulans* cell-free extract were subtracted to correct for the spontaneous mutarotation occurring under assay conditions. One unit of mutarotase activity is defined as the amount of enzyme converting 1 μmol of α-D-glucose at 25°C, pH 7.2, in 1 min. Specific enzyme activities are reported per mg protein. Enzyme activities were measured in at least two biological and up to five technical replicates. Data were analyzed and visualized with SigmaPlot (Systat Software, San Jose, CA, USA), and for each procedure, standard deviations were determined. The significance of changes relative to the control (wild-type) cultures was assessed as above.

Bioinformatic methods

Mutarotases from *Escherichia coli* and human (protein accessions P0A9C3 and Q96C23, respectively) and the C-terminal mutarotase domain of *S. cerevisiae* Gal10p (P04397: residues 356–699) were used as queries in TBLASTN screening (Altschul *et al.* 1997). Ascomycete genes encoding structurally related proteins were extracted from nucleotide databases available at the National Center for Biotechnology Information in the spring of 2015. Gene models and products were deduced manually. To provide an evolutionary basis for phylogenetic analysis, structurally related genes were also identified in fungi classified outside the Dikarya. The 667 peptidic sequences obtained are listed in Fasta format in the Supplementary materials. These proteins were aligned with MAFFT (Katoh and Standley 2013) using the E-INS-i algorithm and a BLOSUM 45 similarity matrix. The alignment was curated with Block Mapping and Gathering using Entropy (Criscuolo and Gribaldo 2010) employing a BLOSUM 45 similarity matrix and a block size of 4. A maximum-likelihood tree was calculated from this curated alignment with PhyML 3.0 applying the WAG substitution model (Guindon *et al.* 2010) and drawn and annotated with FigTree (http://tree.bio.ed.ac.uk/software/figtree). Statistical branch support is provided by approximate likelihood ratio tests (Anisimova and Gascuel 2006) calculated integrally by PhyML using Chi2-based parametrics.

RESULTS

In silico identification of two putative D-galactose mutarotase genes in *Aspergillus nidulans*

The organism in which enzyme-catalyzed mutarotation was first discovered, *Penicillium chrysogenum* (Keilin and Hartree 1952), is closely related with *A. nidulans*. We screened the genome sequences of *A. nidulans* and *P. chrysogenum* for putative homologs of characterized galactose mutarotases. Regardless whether we employed the human enzyme, *Escherichia coli* GalM or the C-terminal domain of the *Saccharomyces cerevisiae* Gal10p bifunctional protein as the query in TBLASTN screening, two genes were identified in *A. nidulans*: an intronless gene at locus AN3184 we have called *galmA*—in concord with the *E. coli* galactose operon gene for mutarotase, *galM*—and a two-exon gene at locus AN3432, *galmB* (NB. Locus identifiers are from annotation version 6 at the Aspergillus Genome Database (Cerqueira *et al.* 2014). These correspond to three *P. chrysogenum* genes at loci Pc20g08410 (51% identical to GalmB at the amino acid level), and Pc13g14400 and Pc20g09570 (60% and 54% identical to GalmA), respectively (NB. *P. chrysogenum* Wisconsin 54–1255 locus identifiers). Unlike in Saccharomycetaceae, Debaryomycetaceae and *Schizosaccharomyces*, neither of these putative mutarotase loci are linked with structural genes of the Leloir pathway, which are scattered around in the genomes of Pezizomycotina. Figure 1 shows the peptide sequences encoded by these five genes aligned with the mutarotase domain of *S. cerevisiae* Gal10p (locus YBR019C), its two single-domain homologs (YNR071C and YHR210C) and the characterized proteins from *E. coli*, *Lactococcus lactis* and *Homo sapiens*. Global similarity is not particulary high but rather, patches of high similarity are alternating with stretches of non-similar sequences. Nevertheless, the six amino acid residues involved in substrate binding and catalysis in the human protein (R82, H107, H176, D243, Q279 and E307) (Thoden *et al.* 2004) are all conserved in the five filamentous fungal proteins as well as in the mutarotase domain of yeast Gal10p.

We investigated the evolutionary relationships between apparent mutarotase homologs in Ascomycota after collecting hundreds of genes encoding mutarotase-like proteins in available genome-sequenced species in all three subphyla. Figure S1 shows a maximum-likelihood phylogeny. Pezizomycotina genomes generally specify more than one putative galactose mutarotase ortholog to the proteins from *A. nidulans* loci AN3184 (*galmA*) or AN3432 (*galmB*), respectively. This is also true for most Basidiomycota taxa (data not shown). In contrast, Taphrinomycotina and Saccharomycotina species (the two other Ascomycota subphyla) generally harbour only one mutarotase gene in their genome, with the notable exception of *S. cerevisiae* (Li *et al.* 2013) and a few species closely related to it. All identified Saccharomycotina and Taphrinomycotina proteins cluster together within the Pezizomycotina AN3432 (GalmB) superbranch, with one exception: a *Debaryomyces hansenii* protein (locus DEHA2D00902g; see Fig.S1, Supporting Information).

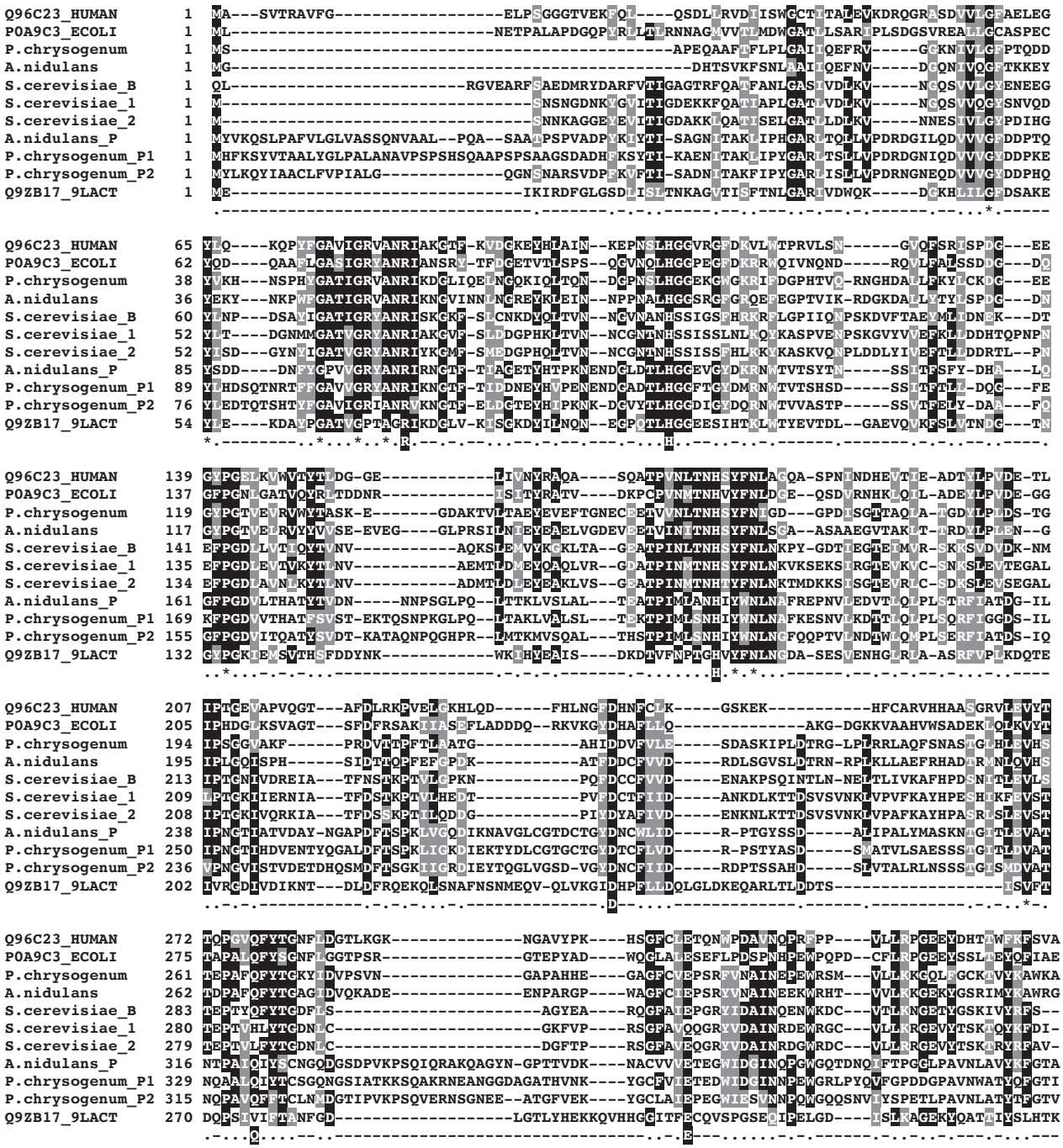

Figure 1. Alignment of fungal mutarotase-like peptides with characterized mutarotases from human, *E. coli* and *L. lactis*. Accession numbers are given for the latter. From the *S. cerevisiae* bifunctional protein Gal10p (S. cerevisiae˙B), only the carboxy-terminal domain (Q356-S699) was used. (S. cerevisiae_1) and (S. cerevisiae_2) are the mutarotase-like proteins specified at loci YNR071C and YHR210C, respectively. The GalmA protein specified at locus AN3184 and the two *Penicillium* proteins specified at loci Pc13g14400 and Pc20g09570 are tagged _P to distinguish them from the GalmB orthologs. The 11 sequences were aligned with MAFFT version 7 using default settings (similarity matrix, Blosum 62). Identical residues or structurally similar residues at conserved positions in six or more sequences are highlighted in shaded backgrounds. For clarity, residues corresponding to human mutarotase R82, H107, H176, D243, Q279 and E307 are given in the consensus line underneath, where asterisks indicate other position-conserved amino acids.

The AN3432 (GalmB) branch also includes the putative mutarotases from the non-dikarya phyla Neocallimastigomycota and Chytridiomycota, and the protein common to all Mucoromycotina species included in the phylogeny. Our phylogenetic analysis could thus not identify a common ancestor to the two distal structural homologs (*galmA* and *galmB*) found in present-day species of Pezizomycotina and certain taxa of Mucoromycotina, which could imply that the two putative mutarotase genes have co-existed before the emergence of Dikarya.

Functional analysis of *galmA* and *galmB*

To investigate whether *galmA* and *galmB* encode physiologically relevant D-galactose mutarotases, the two genes were knocked

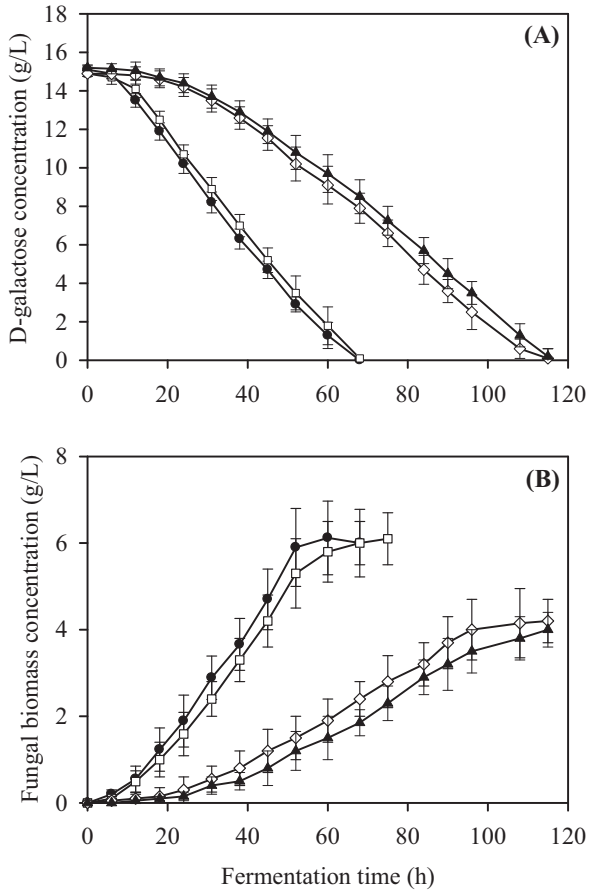

Figure 2. Time profile of residual D-galactose medium concentrations (**A**) as well as growth (**B**) in a batch (submerged) fermentation of the *A. nidulans* wild-type strain (circles) as well as a *galmA* (squares) and *galmB* (diamonds) single deletion mutants and a *galmA*/*galmB* double deletion mutant (triangles) in media initially containing 15 g/L D-galactose. Conidiospores were used as inoculum. D-Galactose was the sole carbon source.

out individually—giving rise to deletion strains Δ*galmA* and Δ*galmB*—as well as simultaneously, resulting in Δ*galmA*/Δ*galmB* double mutants. Strains in which the functional *galm* genes were re-introduced in their respective single gene-deleted backgrounds were also generated. These latter strains carry one or more (i.e. two, three, four or five) gene copies at ectopic loci as revealed by Southern blot analysis (Fig. S2, Supporting Information).

All these *galm* mutant strains were subjected to phenotypic analysis in submerged cultivations. Figure 2 shows that the absence of *galmB* resulted in a considerable decrease in the amounts of D-galactose taken up while biomass formation was significantly ($P < 0.1\%$) delayed as compared to the wild-type control. In contrast, time profiles of biomass formation and D-galactose residual concentrations of the *galmA* deletion mutant cultures were not different ($P < 0.1\%$) from those of the wild-type reference (Fig. 2). Double *galmA*/*galmB* deletion mutants grew as good as the single *galmB* deletion mutants on D-galactose. No growth phenotype was visible on D-glucose (or glycerol or D-fructose—data not shown) in any of the strains investigated, suggesting that our mutants are fully proficient on carbon sources other than D-galactose. Furthermore, the results imply that the putative mutarotase genes are not physiologically relevant for growth on D-glucose.

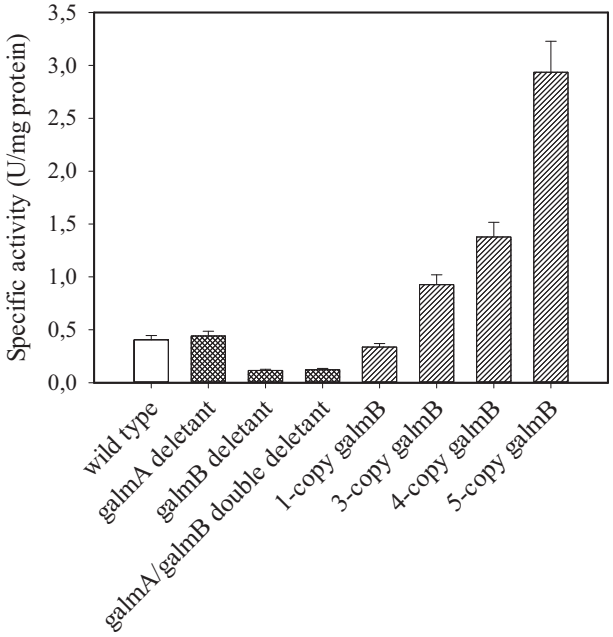

Figure 3. Specific mutarotase activity in the wild type, a *galmA*, a *galmB* and a *galmA*/*galmB* deletion mutant strain as well as a 3-, 4- and 5-copy *galmB* strains. Commercially available α-D-glucopyranose was used as the primary substrate. The shown data are corrected for spontaneous mutarotation in absence of cell-free extract.

GalmB is a physologically relevant D-galactose mutarotase

Although we could not observe any mutarotase phenotype on D-glucose for the *galmB* deletion mutants, we could conveniently test aldose mutarotation by means of a customized enzyme assay that actually measures glucose mutarotation (Bouffard, Rudd and Adhya 1994). Assaying cell-free extracts from D-galactose-grown or D-glucose-grown biomass of the *galmB* single- and *galm* double *A. nidulans* mutants, the observed mutarotation did not significantly ($P < 0.1\%$) exceed the spontaneous anomer conversion found in the reaction buffer or in heat-inactivated cell-free extracts. On the contrary, *galmB* multicopy strains displayed significantly ($P < 0.1\%$) higher enzyme activities than the wild type, increasing with the copy number (Fig. 3). Nevertheless, overexpressing (multicopy) mutants of *galmB* did not affect the growth or sugar uptake, implying that catalyzed mutarotation is not rate limiting for D-galactose catabolism in the wild-type background under the tested conditions (Fig. 4). On the other hand, overexpression of *galmA* from multiple copies did not have any physiological effect on growth on D-galactose or on D-glucose mutarotation either. Importantly, both *galm* genes were expressed under the growth conditions used in this study on D-glucose (not shown) as well as on D-galactose (Fig. 5). No physiological effects were observed from *galm* overexpression on any of the other growth substrates tested (D-glucose, D-fructose, glycerol; data no shown).

DISCUSSION

D-Galactose catabolism in *Aspergillus nidulans* proceeds via two parallel biochemical routes. Thus, to validate the genuine contribution of mutarotase in the catabolism of D-galactose via the

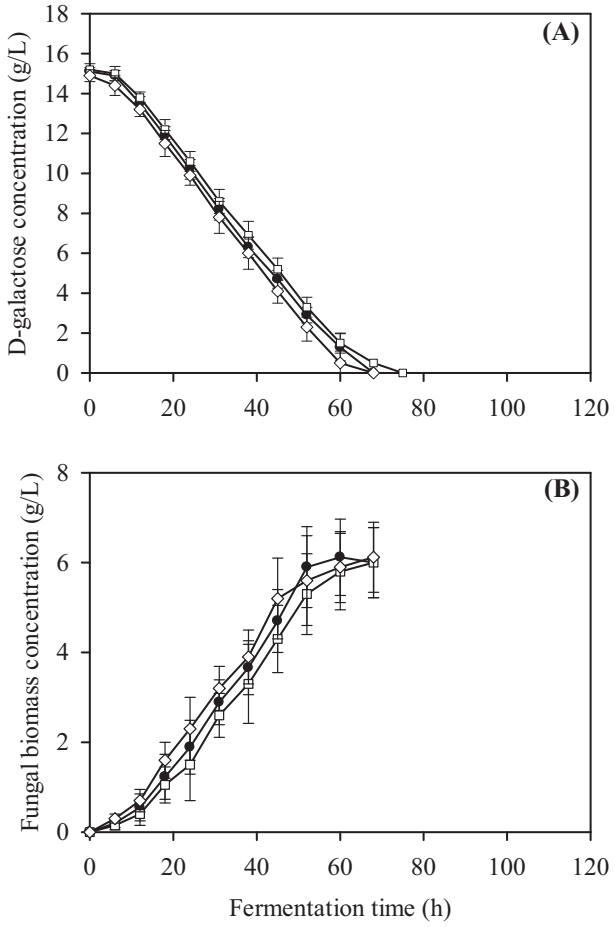

Figure 4. Time profile of residual D-galactose medium concentrations (**A**) as well as growth (**B**) in a batch (submerged) fermentation of the *A. nidulans* wild-type strain (circles) as well as a *galmA* 2-copy (squares) and *galmB* 3-copy (diamonds) strains mutant in media initially containing 15 g/L D-galactose. Conidiospores were used as inoculum. D-Galactose was the sole carbon source. Monocopy complemented strains essentially behaved like the wild-type control R21.

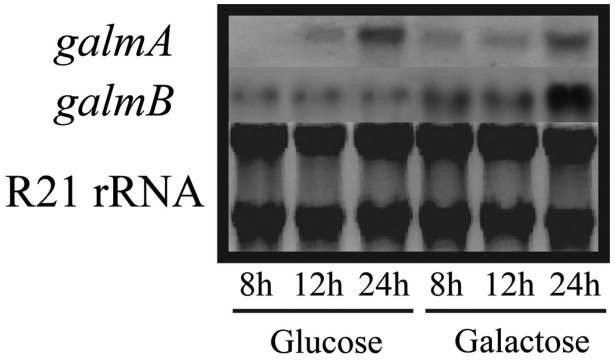

Figure 5. Transcript analysis of the *A. nidulans galmA* and *galmB* genes in the wild-type strain. rRNA (28S and 18S) was visualized with ethidium bromide and is shown at the bottom as a control for RNA sample quality and quantity.

ubiquitous Leloir route, the alternative oxidoreductive pathway has to be inactive. The oxidoreductive pathway is facilitated when ammonium is present in the growth medium as the nitrogen source, whereas it is inactive when nitrate is used (Fekete *et al.* 2004; Alam and Kaminskyj 2013). This—still unexplained—attribute of the *A. nidulans* oxidoreductive pathway allowed to switch off the oxidoreductive path by physiological means, using the standard synthetic AMM medium (Pontecorvo *et al.* 1953) that contains nitrate as the nitrogen source. However, expression of either *galm* genes on D-glucose or D-galactose was not influenced by the nitrogen source in the growth medium (data not shown).

Manipulation of the *galmA* copy number, i.e. deletion or overexpression of the gene did not influence specific mutarotase activity. From the alignment (Fig. 1) it appeared that GalmA has an N-terminal extention compared to GalmB. *Aspergillus nidulans* GalmA carries a predicted, 23-residue long signal peptide for secretion if one takes the second ATG of the open reading frame at the intronless locus AN3184 as the start codon (NB. The translated peptide would be 403 residues long rather than 447, as predicted by autoannotation). We verified all 315 GalmA-like proteins collected for the phylogeny and 301 have a clear secretion signal predicted either with TargetP or SignalP (Emanuelsson *et al.* 2007), with signal peptides typically varying in length from 15 to 26 residues. This suggests that GalmA is not intracellular which could explain why *galmA* deletion does not affect galactose catabolism. However, we cannot exclude the possibility that GalmA activity is not detectable with our β-D-glucose dehydrogenase-based assay due to an inability of GalmA to act on glucose anomers. The *Saccharomyces cerevisiae* Gal10p mutarotase domain *in vitro* interconverts the anomers of D-galactose 100 times faster than those of D-glucose, the latter's conversion rate being not much higher than the spontaneous mutarotation rate in the assay buffer (Scott and Timson 2007). This could also suggest that glucose anomerization is merely a gratuitous side activity of (some of) the fungal galactose 1-epimerases. In accord with this, deletion or overexpression (from its own promoter) of either of the *A. nidulans galm* genes did not have an effect on the utilization of D-glucose as the carbon source. Although hexokinase (glucokinase) phosphorylates both anomers at C6 (Wurster and Hess 1974) and glucose-6-phosphate (G6P) aldose-ketose isomerase is capable of G6P anomerization (Schray *et al.* 1973), fungi, plants and bacteria possess a specialized mutarotase for G6P that acts on C6-phosphorylated sugars only (Wurster and Hess 1972; Graille *et al.* 2006). Indeed in *S. cerevisiae*, expression of the complete galactose catabolism gene cluster, including that of the bifunctional *GAL10* gene that encodes the galactose mutarotase activity, is glucose repressed (Sellick, Campbell and Reece 2008). In *A. nidulans*, G6P 1-epimerase (EC 5.1.3.15) is specified by locus AN10222 and this enzyme may render galactose mutarotase (GalmB) irrelevant for growth on D-glucose.

In summary, while we can only speculate about a physiological role of GalmA, an *A. nidulans galmB* deletion mutant displays a profound growth effect on D-galactose under conditions that inhibit the alternative (oxidoreductive) pathway, unlike in *E. coli* where GalM deletion does not result in a growth phenotype (Bouffard, Rudd and Adhya 1994). On the other hand, in *Kluyveromyces lactis*, deletion of Gal10 leads to a complete loss of capacity to grow on D-galactose, strongly suggesting that the oxidoreductive pathway does not operate in this yeast. Growth on D-galactose is not restored upon re-introduction of the DNA coding for the (functional) N-terminal UDP-galactose 4-epimerase domain of Gal10 (Fukasawa *et al.* 2009). In physiological terms, the loss-of-function *A. nidulans* D-galactose mutarotase phenotype is thus somewhere in the middle between *E. coli* and *K. lactis*.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

ACKNOWLEDGEMENT

The authors are grateful to Dr Norbert Ág, Nikoletta Lipcsák, Dániel Tamasik and Enikő Szatmári for their contributions.

FUNDING

Research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008 and by the Hungarian Scientific Research Fund (OTKA NN116519) to LK, who is also a recipient of a Bólyai János Research Scholarship (BO/00548/14). László Kulcsár is a recipient of a grant from the New National Excellence Program of the Ministry of Human Capacities (ÚNKP-16-3).

Conflicts of interest. None declared.

REFERENCES

Alam MK, Kaminskyj SGW. *Aspergillus* galactose metabolism is more complex than that of *Saccharomyces*: the story of $GalD^{GAL7}$ and $GalE^{GAL1}$. *Botany* 2013;**91**:467–77.

Altschul SF, Madden TL, Schäffer AA *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;**25**:3389–402.

Anisimova M, Gascuel O. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst Biol* 2006;**55**:539–52.

Bouffard GG, Rudd KE, Adhya SL. Dependence of lactose metabolism upon mutarotase encoded in the *gal* operon in *Escherichia coli*. *J Mol Biol* 1994;**244**:269–78.

Cerqueira GC, Arnaud MB, Inglis DO *et al.* The Aspergillus Genome Database: multispecies curation and incorporation of RNA-Seq data to improve structural gene annotations. *Nucleic Acids Res* 2014;**42**:D705–10.

Clutterbuck AJ. Aspergillus nidulans. In: King RC (ed) *Handbook of Genetics*, vol 1. New York: Plenum Press, 1974; 447–510.

Conway LP, Voglmeir J. Functional analysis of anomeric sugar kinases. *Carbohydr Res* 2016;**432**:23–30.

Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evol Biol* 2010;**10**:210.

Emanuelsson O, Brunak S, von Heijne G *et al.* Locating proteins in the cell using TargetP, SignalP, and related tools. *Nat Protoc* 2007;**2**:953–71.

Fekete E, Karaffa L, Sándor E *et al.* Regulation of formation of the intracellular β-galactosidase activity of *Aspergillus nidulans*. *Arch Microbiol* 2002;**179**:7–14.

Fekete E, Karaffa L, Sándor E *et al.* The alternative D-galactose degrading pathway of *Aspergillus nidulans* proceeds via L-sorbose. *Arch Microbiol* 2004;**181**:35–44.

Fekete E, Seiboth B, Kubicek CP *et al.* Lack of aldose-1 epimerase in *Hypocrea jecorina* (anamorph *Trichoderma reesei*): a key to cellulase gene expression on lactose. *P Natl Acad Sci USA* 2008;**105**:7141–6.

Flipphi M, Sun J, Robellet X *et al.* Biodiversity and evolution of primary carbon metabolism in *Aspergillus nidulans* and other *Aspergillus* spp. *Fungal Genet Biol* 2009;**46**: S19–44.

Frey PA. The Leloir pathway: a mechanistic imperative for three enzymes to change the stereochemical configuration of a single carbon in galactose. *FASEB J* 1996;**10**:461–70.

Fukasawa T, Sakurai H, Nogi Y *et al.* Galactose transporters discriminate steric anomers at the cell surface in yeast. *FEMS Yeast Res* 2009;**9**:723–31.

Graille M, Baltaze JP, Leulliot N *et al.* Structure-based functional annotation: yeast *ymr099c* codes for a D-hexose-6-phosphate mutarotase. *J Biol Chem* 2006;**281**:30175–85.

Grossiord BP, Luesink EJ, Vaughan EE *et al.* Characterization, expression, and mutation of the *Lactococcus lactis galPMKTE* genes, involved in galactose utilization via the Leloir pathway. *J Bacteriol* 2003;**185**:870–8.

Gruber F, Visser J, Kubicek CP *et al.* Cloning of the *Trichoderma reesei pyrG* gene and its use as a homologous marker for a high-frequency transformation system. *Curr Genet* 1990;**18**:447–51.

Guindon S, Dufayard JF, Lefort V *et al.* New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 2010;**59**:307–21.

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;**30**:772–80.

Keilin D, Hartree EF. Biological catalysis of mutarotation of glucose. *Biochem J* 1952;**50**:341–8.

Li S, Ha SJ, Kim HJ *et al.* Investigation of the functional role of aldose 1-epimerase in engineered cellobiose utilization. *J Biotechnol* 2013;**168**:1–6.

Nayak T, Szewczyk E, Oakley CE *et al.* A versatile and efficient gene-targeting system for *Aspergillus nidulans*. *Genetics* 2006;**172**:1557–66.

Pontecorvo G, Roper JA, Hemmons LM *et al.* The genetics of *Aspergillus nidulans*. *Adv Genet* 1953;**5**:141–238.

Sambrook J, Russell DW. *Molecular Cloning: a Laboratory Manual*, 3rd edn. New York: Cold Spring Harbor Laboratory, 2001.

Schray KJ, Benkovic SJ, Benkovic PA *et al.* Catalytic reactions of phosphoglucose isomerase with cyclic forms of glucose 6-phosphate and fructose 6-phosphate. *J Biol Chem* 1973;**248**:2219–24.

Scott A, Timson DJ. Characterization of the *Saccharomyces cerevisiae* galactose mutarotase/UDP-galactose 4-epimerase protein, Gal10p. *FEMS Yeast Res* 2007;**7**:366–71.

Sellick CA, Campbell RN, Reece RJ. Galactose metabolism in yeast—structure and regulation of the Leloir pathway enzymes and the genes encoding them. In: Jeon KW (ed.). *International Review of Cell and Molecular Biology*. London: Academic, 2008;111–50.

Slot JC, Rokas A. Multiple *GAL* pathway gene clusters evolved independently and by different mechanisms in fungi. *P Natl Acad Sci USA* 2010;**107**:10136–41.

Thoden JB, Timson DJ, Reece RJ *et al.* Molecular structure of human galactose mutarotase. *J Biol Chem* 2004;**279**:23431–7.

Tilburn J, Scazzocchio C, Taylor GG *et al.* Transformation by integration in *Aspergillus nidulans*. *Gene* 1983;**26**:205–21.

Wurster B, Hess B. Glucose-6-phosphate-1-epimerase from baker's yeast. A new enzyme. *FEBS Lett* 1972;**23**:341–4.

Wurster B, Hess B. Anomeric specificity of enzymes of D-glucose metabolism. *FEBS Lett* 1974;**40**:S105–11.

Yu JH, Hamari Z, Han KH *et al.* Double-joint PCR: a PCR-based molecular tool for gene manipulations in filamentous fungi. *Fungal Genet Biol* 2004;**41**:973–81.