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Characterization of a second physiologically relevant lactose permease gene (*lacpB*) in *Aspergillus nidulans*

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In *Aspergillus nidulans*, uptake rather than hydrolysis is the rate-limiting step of lactose catabolism. Deletion of the lactose permease A-encoding gene (*lacpA*) reduces the growth rate on lactose, while its overexpression enables faster growth than wild-type strains are capable of. We have identified a second physiologically relevant lactose transporter, LacpB. Glycerol-grown mycelia from mutants deleted for *lacpB* appear to take up only minute amounts of lactose during the first 60 h after a medium transfer, while mycelia of double *lacpA*/*lacpB*-deletant strains are unable to produce new biomass from lactose. Although transcription of both *lacp* genes was strongly induced by lactose, their inducer profiles differ markedly. *lacpA* but not *lacpB* expression was high in D-galactose cultures. However, *lacpB* responded strongly also to β-linked glucopyranose dimers cellobiose and sophorose, while these inducers of the cellulolytic system did not provoke any *lacpA* response. Nevertheless, *lacpB* transcript was induced to higher levels on cellobiose in strains that lack the *lacpA* gene than in a wild-type background. Indeed, cellobiose uptake was faster and biomass formation accelerated in *lacpA* deletants. In contrast, in *lacpB* knockout strains, growth rate and cellobiose uptake were considerably reduced relative to wild-type, indicating that the cellulose and lactose catabolic systems employ common elements. Nevertheless, our permease mutants still grew on cellobiose, which suggests that its uptake in *A. nidulans* prominently involves hitherto unknown transport systems.

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INTRODUCTION

The dairy industry generates prodigious amounts of liquid waste (Gänzle *et al.*, 2008). Some 85 % of the components of the milk destined for cheese manufacture are eventually discarded as a watery lactose-rich by-product called whey (Marwaha & Kennedy, 1988). Generated at over 1.5 million tonnes per year worldwide (Roelfsema *et al.*, 2010), raw, untreated whey poses huge environmental challenges, as current and past wastewater-treatment technologies are expensive. An alternative to disposal is utilizing the whey residue in downstream (industrial) processes by which value-added products are or can be manufactured (Panesar & Kennedy, 2012). A prime example is industrial-scale fermentation biotechnology for which whey is traditionally considered a cheap and abundant growth substrate and nitrogen source for micro-organisms, fungi in particular (Coghill & Moyer, 1947; Silva *et al.*, 2009; Panesar *et al.*, 2006; Kumari *et al.*, 2011; Aghcheh & Kubicek, 2015).

Lactose (1,4-*O*-β-D-galactopyranosyl-D-glucose; milk sugar) is the main carbohydrate in whey. Mammalian milk is the only source of lactose in nature. For most micro-organisms that can hydrolyse it into D-glucose and D-galactose, lactose is a slowly assimilated, gratuitous carbon source not encountered in their natural habitats. This characteristic considerably facilitates the (over)production of secondary metabolites and hydrolytic enzymes by saprophytic- and plant-pathogenic fungi that apparently grow under laboratory conditions on lactose by chance rather than by design. Operation of the *LAC* regulon of the lactose-fermenting yeast *Kluyveromyces lactis* is a paradigm for transcriptional control in lower eukaryotes (see, for example: Cardinali *et al.*, 1997; Baruffini *et al.*, 2006; Rigamonte *et al.*, 2011). Nevertheless, several key aspects of fungal lactose metabolism in less substrate-adapted ascomycete filamentous fungi (including potent cell factories) are poorly understood (see, for example: Seiboth *et al.*,

Abbreviations: DCW, dry cellular weight; MFS, major facilitator superfamily.

Two supplementary tables and two supplementary figures are available with the online Supplementary Material.

2007; Karaffa *et al.*, 2013). To optimize fermentation processes that use whey residue, and to further its use in second-generation biofuel generation and its removal from contaminated soil and water (bioremediation), we have studied lactose catabolism in several filamentous fungi including the genetic model *Aspergillus nidulans*, a soil-borne saprophyte.

Two strategies have been described for the catabolism of lactose in fungi: extracellular hydrolysis and subsequent uptake of the resulting monomers, i.e. D-glucose and D-galactose, and uptake of the disaccharide followed by intracellular hydrolysis (reviewed by, for example, Seiboth *et al.*, 2007). In analogy with the *K. lactis* lactose assimilation system (e.g. Gödecke *et al.*, 1991; Diniz *et al.*, 2012), we identified and characterized two clustered, divergently transcribed genes in *A. nidulans*, encoding an intracellular β-galactosidase of the glycoside hydrolase family 2 (*bgaD*) and a lactose permease belonging to the major facilitator superfamily (MFS) of transmembrane proteins (*lacpA*) (Fekete *et al.*, 2012). These two genes were expressed to basal levels in carbon-derepressed (*creA*^{d}) mutant backgrounds, even when the strongly repressing sugar D-glucose was the only growth substrate present. By creating deletion mutants, we provided evidence that *bgaD* is the only hydrolase in *A. nidulans* that acts on the chromogenic substrate X-Gal – generally regarded as a typical albeit artificial β-galactosidase substrate. Moreover, we demonstrated that LacpA – the first physiologically relevant, fungal lactose permease described outside the *Saccharomycetales* – mediates high-affinity uptake of lactose. Such a β-galactosidase/lactose permease gene cluster was found conserved in at least 15 other filamentous ascomycetes (*Pezizomycotina*) (Fekete *et al.*, 2012).

Furthermore, evidence was provided that transport rather than hydrolysis is the limiting step of lactose catabolism in *A. nidulans*, as overexpression of *lacpA* allows multiple copy transformants to grow considerably faster on the disaccharide than wild-type strains can. Although LacpA is responsible for a considerable part of the lactose uptake in this fungus, *lacpA* knockout strains still grow on it (Fekete *et al.*, 2012), which is indicative that at least one additional uptake system must be operative. In this report, we identify and functionally analyse a second physiologically relevant lactose permease gene in *A. nidulans*, which we have named *lacpB*.

METHODS

***A. nidulans* strains, media and culture conditions.** *A. nidulans* strains and transformants used in this study are listed in Table S1 (available in the online Supplementary Material). Minimal media (AMM2) for shake-flask and bioreactor cultivations (the latter henceforth referred to as fermentations) were formulated and inoculated as described by Fekete *et al.* (2002). Vitamins and other supplements were added from sterile stock solutions. Carbon sources were used at 1.5 % (w/v) initial concentration unless stated differently. 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 medium in a rotary shaker at 200 r.p.m. Fermentations were carried out in a 2.5 l glass vessel (Sartorius) with a culture volume of 2 l, and equipped with one six-blade Rushton disc turbine impeller. Operating conditions were pH 6.5, 37 °C and 0.5 vvm (volumes of air per volume of liquid min^{-1}). The dissolved oxygen level was maintained at 20 % saturation and was controlled by means of the agitation rate. To minimize medium loss, the waste gas was cooled in a reflux condenser connected to an external cooling bath (4 °C) before exiting the system.

For induction experiments (also referred to as expression or transcript analysis), replacement cultures were used for which mycelia were pregrown for 24 h in AMM2 medium containing glycerol as the carbon source, and harvested by filtration over a sintered glass funnel. After thoroughly washing the biomass with cold sterile water, mycelia were transferred to flasks with carbon-free, fresh AMM2 and were pre-incubated for 1 h in a rotary shaker at 200 r.p.m., after which the carbon sources to be tested were added to the cultures in final concentrations up to 25 mM. Samples were taken after 3, 6 and 12 h of further incubation to assess inductory ability. Preliminary trails had established that 3 h of contact is the time lapse in which maximal induced transcript levels were achieved, with a minimal variation in the biomass concentration.

Lactose-uptake experiments. Conidiospores were inoculated overnight on 1 % (v/v) glycerol as the carbon source. Grown mycelia were transferred first to AMM2 medium containing lactose (15 g l^{-1}) as a sole carbon source for 24 h. Mycelia were subsequently harvested by gentle filtration over sterile cheese cloth, thoroughly washed with carbon-free AMM2 and resuspended in 500 ml Erlenmeyer flasks containing AMM2 to yield a final biomass concentration of 1 g l^{-1}. Lactose was administered to final concentrations of 0.2, 0.5 and 2 mM, and the cultures were incubated for a further 6 h in a rotary shaker (37 °C, 200 r.p.m.) to monitor sugar consumption. Samples were withdrawn at regular intervals, cellular debris was spun down in an Eppendorf centrifuge (10 000 ***g***, 5 min), and residual lactose in the supernatant was determined by HPLC (see below). D-Glucose in a final concentration of 2 mM was used for the control cultures. Biomass-corrected uptake was expressed in μmol [g dry cellular weight (DCW)]$^{-1}$. Specific uptake rates were calculated from the specific uptake plotted against time, and were expressed in μmol lactose min^{-1} (g DCW)$^{-1}$.

Classical genetic techniques and transformation. Conventional genetic techniques were employed to exchange markers by meiotic recombination (Clutterbuck, 1974). Progeny of sexual crosses were tested for known auxotrophies using standard techniques. *A. nidulans* transformations were performed basically as described by Tilburn *et al.* (1983), using Glucanex (Novozymes) as cell-wall lysing agent. 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 over nylon mesh and thoroughly washed with sterile distilled water. Excess liquid was removed by squeezing the mycelia between paper sheets, and the biomass was rapidly frozen in liquid nitrogen. For nucleic acid isolation, frozen biomass was ground to a dry powder using a liquid nitrogen-chilled mortar and pestle. 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). RNA quality was routinely evaluated upon electrophoresis in native 2 % agarose gels. The concentration and purity of the RNA samples were determined by using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

Northern and Southern blot analysis. Standard procedures (Sambrook & Russell, 2001) were used for the quantification, denaturation, gel separation and nylon blotting of nucleic acids, and the

hybridization of the membranes. Agarose gels were charged with 5 μg DNA/RNA per slot. Probes were digoxigenin-labelled using a PCR DIG probe synthesis kit (Roche Applied Science) primed with gene-specific oligonucleotides (Table S2) off R21 genomic DNA. Hybridization was visualized with Lumi-Film chemiluminescent detection film (Roche Applied Science). cDNA was synthesized from 1 μg DNase I-treated total RNA using oligo(dT) as a primer and Moloney murine leukaemia virus reverse transcriptase.

Generation of knockout mutant strains. A gene deletion cassette was constructed *in vitro* according to the double-joint PCR method (Yu *et al.*, 2004). The cassette consisted of 840 bp of the terminal, non-coding regions of *A. nidulans lacpB*, flanking the functional *A. fumigatus pyroA* gene involved in pyridoxine biosynthesis (Nayak *et al.*, 2006). The oligonucleotide primers used are listed in Table S2. Protoplasts of *A. nidulans* pyridoxine-auxotroph strain TN02A3 were transformed with 10 μg of the linear deletion cassette. This transformation host greatly facilitates the obtention of gene knockouts due to the absence of a functional non-homologous end-joining machinery in the *nkuA* deleted background (Nayak *et al.*, 2006). Pyridoxine-prototroph transformants were probed for the absence of *lacpB* coding sequences by PCR, primed off genomic DNA using gene-specific primers.

For the creation of the double transporter mutant (*lacpA*/*lacpB*), the same *in vitro lacpB* replacement construct as described above was introduced into one of the pyridoxine-auxotroph Δ*lacpA*/Δ*nkuA* strains (Fekete *et al.*, 2012). Selected pyridoxine-prototroph, double-deletant strains were verified by Southern blot analysis and then crossed out to remove the *nkuA* deletion. Throughout this work, two independent double transporter mutants were tested in parallel.

Reintroduction of *lacpB* into gene-deleted backgrounds. A characterized first generation deletant of *lacpB* was crossed with strain RJMP155.55. Pyridoxine-prototroph and riboflavin-auxotroph offspring were verified by PCR for the presence of the *nkuA* gene. A functional *lacpB* gene was amplified off *A. nidulans* R21 genomic DNA using specific primers (Table S2). 10 μg amplification product was co-transformed with 1 μg pTN2 (carrying the *A. fumigatus riboB* gene encoding a protein involved in riboflavin biosynthesis; Nayak *et al.*, 2006) into one of the Δ*nkuA*-cured, second generation gene-deleted strains. Among the riboflavin-prototroph transformants, the presence of the reintroduced gene was probed by PCR. The *lacpB* copy number was subsequently estimated by Southern blot analysis, and selected strains that had reacquired functional *lacpB* in one or more copies were phenotypically characterized.

Analytical methods. DCW was determined from 10 ml culture aliquots. The biomass was harvested and washed on a preweighted glass wool filter by suction filtration, washed with cold tap water and the filter dried at 80 °C until constant weight. Dry weight data reported in Results are the means of the two separate measurements, which never deviated by more than 14 %. D-Glucose, D-galactose and lactose were determined by HPLC with refractive index detection as described by Fekete *et al.* (2002).

Reproducibility. The kinetic data (time profiles for the biomass and the carbon source) presented are the means of three independent fermentations (biological replicates). Expression datasets reported were performed with at least two biological replicates and up to five technical replicates, and representative images are shown. Lactose-uptake data were obtained after experimentation with biological triplicates. Data were analysed and visualized with SigmaPlot (Jandel Scientific), and for each procedure, SDs were determined. The significance of changes in biomass and in residual lactose concentration in the growth medium of mutant or complemented deletant strains, relative to the control cultures, was assessed using Student's *t*-test with probability (*P*) values given in Results.

Chemicals. Except where specified, chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich.

Bio-informatics methods. *A. nidulans* lactose permease A (LacpA) (Fekete *et al.*, 2012) was used as the query in TBLASTN mining of genes encoding structurally related MFS transmembrane proteins in some three dozen species of the genus *Aspergillus*, from nucleotide databases available at the National Center for Biotechnology Information and the US Department of Energy Joint Genome Institute. Gene models and products were deduced manually. The 92 protein sequences most similar to *A. nidulans* LacpA were aligned with MAFFT version 7 (Katoh & Toh, 2010) using the G-INS-i algorithm (trained on global homology) and a BLOSUM 45 similarity matrix. Protein input is available from the corresponding author upon request. The alignment was curated with Block Mapping and Gathering using Entropy (BMGE version 1.12; Criscuolo & Gribaldo, 2010) employing a BLOSUM 35 similarity matrix and a block size of 3, yielding 477 informative residues per protein. A maximum-likelihood tree (Fig. S1) was then calculated with PhyML version 3.0 applying the WAG substitution model (Guindon *et al.*, 2010) and drawn with FigTree (available at http://tree.bio.ed.ac.uk/software/figtree). Approximate likelihood ratio tests (Anisimova & Gascuel, 2006) were calculated integrally by PhyML using Chi2-based parametrics; aLRT values (0–1) are given at the connecting nodes in the tree.

RESULTS

In silico identification and expression verification of putative lactose permease genes

The genome of *A. nidulans* harbours over 100 predicted genes that encode transmembrane proteins belonging to the sugar transporter family (Pfam00083) of the MFS (Wortman *et al.*, 2009). To track down structurally related permeases, we examined the phylogenetic relations amongst a selection of *Aspergillus* MFS proteins (Fig. S1). The genes for the LacpA orthologues and for the seven structural paralogue proteins most similar to *A. nidulans* LacpA (locus identifier AN3199) were mined from publicly accessible nucleotide databases upon a TBLASTN search (Altschul *et al.*, 1997), and the respective gene models and protein products were subsequently deduced. The resulting 92 proteins were used as input to build a maximum-likelihood tree. Lactose permease LacpA (protein_1 in Fig. S1) is specified in only 14 genomes, while a closely related protein (protein_2) is encoded in all but 3 of the 31 genomes. Nevertheless, no species were found to harbour all selected MFS genes: *Aspergillus ustus* and *Aspergillus versicolor* carry six of the paralogue genes. However, *Aspergillus glaucus* and *Aspergillus rubrum* (section *Aspergillus*) lacked all eight (not shown). The three structurally related *A. nidulans* MFS proteins mined among the seven most similar *Aspergillus* paralogues of LacpA, specified by genes at the annotated loci AN1577 (protein_2), AN6831 (protein_3) and AN2814 (protein_4), were selected as candidate lactose permeases to subject to a biological validation.

The expression of the three candidate genes in response to the presence of lactose and – as a negative control of expression – D-glucose was addressed by reverse transcriptase PCR. As shown in Fig. 1, the gene specified at locus AN2814 was well expressed in the presence of lactose 6 h after a medium transfer of glycerol-grown mycelia, compared to glucose. However, the gene at locus AN1577 gave a much weaker signal on lactose, while the third paralogue gene (locus AN6831) appears to be modestly transcribed on glucose rather than specifically on lactose. Moreover, the related fungus *Penicillium chrysogenum* – *Penicillium* is the sister genus of *Aspergillus* in the family of the *Aspergillaceae* – reportedly has the intracellular GH2 β-galactosidase orthologue of *A. nidulans* BgaD but its genome does not specify transporters orthologous to LacpA and that encoded at *A. nidulans* locus AN1577 (Jónás *et al.*, 2014). In conclusion, *in silico* analysis of the *A. nidulans* genome sequences followed by a restricted test of expression of three selected candidates suggested that the gene at locus AN2814 encodes a LacpA-paralogue MFS protein that may contribute to uptake of lactose by *A. nidulans*. We named this putative second lactose permease gene, *lacpB*.

Confirmation that *lacpB* encodes a lactose permease and functional analysis of the gene

To verify that *lacpB* indeed encodes a functional lactose permease, the gene was knocked out. *lacpB* deletants and strains in which the functional gene was reintroduced in the gene-deleted background carrying one or more (i.e. two and five) copies at ectopic loci were subjected to phenotypic analysis. As Fig. 2 shows, *lacpB* deletion led to a sharp decrease in the amounts of lactose taken up when compared to the wild-type control strain, and no (new) biomass was formed before the third day of fermentations with lactose as the sole carbon source. However, no growth phenotype was visible on D-glucose or D-galactose (data not shown). Somewhat unexpectedly, lactose uptake as

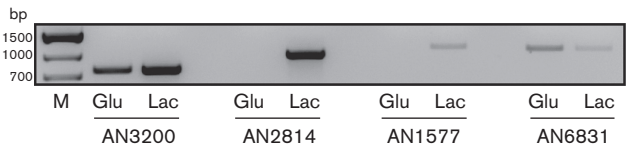

Fig. 1. Expression of three putative lactose permease genes (AN2814, AN1577, AN6861) and the reference gene (AN3200) in response to lactose visualized by semiquantitative reverse transcriptase PCR. The strongly repressing carbon source D-glucose serves as a control condition: *lacpA* and *bgaD* expression are repressed by glucose even when lactose is also present in the growth medium. The reference gene at locus AN3200 – located between *lacpA* and *bgaD* – was previously shown to be constitutively expressed to low levels under a number of conditions, including on D-glucose (Fekete *et al.*, 2012). Glu, D-Glucose; Lac, lactose; M, DNA size markers.

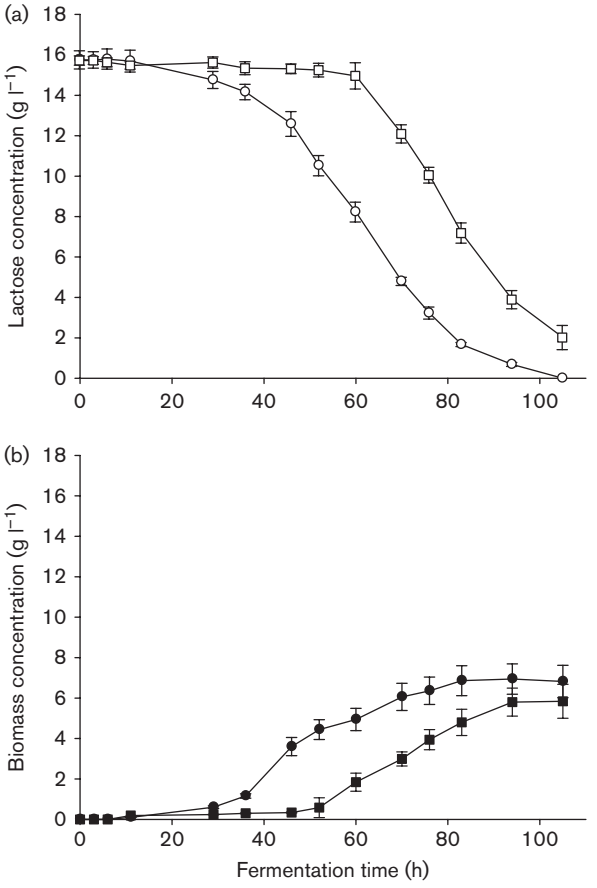

Fig. 2. Time profile of residual lactose concentrations (a), as well as growth (b), in a batch fermentation of the *A. nidulans* wild-type strain (circles) and a *lacpB* deletion mutant (squares) in media initially containing 15 g lactose l^{-1}. Conidiospores were used as the inoculum. Lactose was the sole carbon source.

well as fungal biomass formation set off approximately 3 days after inoculation at a rate comparable to that of the wild-type strain at the rapid growth stage of the cultivation, and continued until the available lactose was fully consumed (Fig. 2). Nevertheless, final maximal biomass concentrations remained significantly ($P<0.1$ %) lower than in the reference culture. This pattern of delayed carbon uptake and growth during lactose fermentation of the *lacpB*-deletant strains appeared to occur regardless of the initial substrate concentration in the range of 1 to 20 g l^{-1} ($\sim$3–58 mM; data not shown). At 50 and 100 g l^{-1} ($\sim$0.15 and 0.3 M) of initial lactose concentrations – which is the usual range found in mammalian milk – even the wild-type strain appeared growth inhibited, a phenomenon that may indicate that *A. nidulans* has little tolerance against high concentrations of lactose at the stage of germination (Fig. S2).

Uptake is rate-limiting for wild-type lactose assimilation in *A. nidulans* (Fekete *et al.*, 2012). Investigation of the complemented *lacpB* knockout strains revealed that the copy

number of *lacpB* was proportional to the efficiency of lactose uptake and subsequent biomass formation (Fig. 3). Since single-copy retransformants behaved essentially identically to the wild-type strain, the results in strains with multiple copies of *lacpB* implied that LacpB is physiologically relevant. Multi-copy strains displayed a significantly ($P<1$ %) enhanced lactose-uptake rate, as evident from the accelerated disappearance of lactose from the growth medium. Concomitantly, biomass formation was also significantly ($P<1$ %) faster and the growth rate increased with the increasing number of *lacpB* copies. As a consequence, final biomass concentrations achieved by the multi-copy strains were significantly ($P<1$ % for the 2-copy strain, $P<0.1$ % for the 5-copy strain) higher than those of the wild-type or the (monocopy) retransformant strains.

To demonstrate that lactose uptake is affected in *lacpB*-deleted strains, pre-grown mycelia of the reference as well as the mutant strains were incubated with low concentrations (0.2, 0.5, 2 mM) of lactose (Fig. 4). At each concentration, specific lactose uptake of the wild-type strain was higher than that of deletant, but lower compared to the multi-copy mutants (Fig. 4). Moreover, lactose uptake was significantly ($P<1$ %) faster in the strain that carried five copies of the *lacpB* gene compared to the one harbouring two copies.

To determine whether the contribution of LacpB to the overall uptake of lactose varied with the external substrate concentration, lactose uptake was studied in the wild-type reference strain and a *lacpB*-deletant strain, as well as in the 2-copy and 5-copy *lacpB*-retransformant strains (Table 1). The data show that while lactose uptake of the *lacpB*-deletant strain at 0.2 and 0.5 mM (initial) external lactose concentrations was approximately 14 and 11 % of that of the wild-type strain at those concentrations, respectively, it was about 22 % at 2 mM. All these observations are direct evidence that *lacpB* encodes a physiologically relevant lactose permease in *A. nidulans*. Moreover, LacpB appears crucial to initiate and sustain uninhibited growth on lactose at concentrations up to 20 g l^{-1} without a long lag phase.

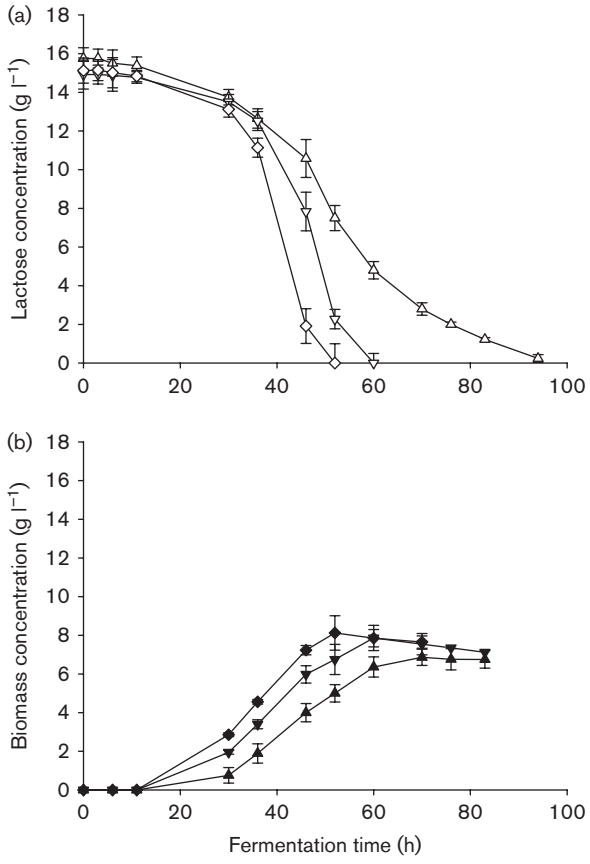

Fig. 3. Kinetics of lactose uptake (a) and biomass formation (b) of the monocopy retransformant strain AOEF011.1 (up-pointed triangles) compared to the 2-copy (down-pointed triangles) and 5-copy (diamonds) *lacpB* strains AOEF011.9 and AOEF011.7, respectively. Note that the monocopy retransformant essentially behaves like wild-type control strain R21.

Lactose uptake is mediated by two permeases in *A. nidulans*

In a previous publication, we demonstrated that the *A. nidulans lacpA* gene encodes a lactose permease that is physiologically relevant albeit only responsible for part of the total uptake (Fekete *et al.*, 2012). The new data on the function of the paralogue permease LacpB described above prompted us to investigate whether it may account for the remaining uptake potential in *lacpA*-deletant strains. To this end, *lacpB* was deleted in a *lacpA*-negative background, giving rise to *lacpA/B* double lactose permease mutants. Phenotypic analysis of the strains revealed that conidial germination was completely inhibited in liquid media with lactose as the sole carbon source at concentrations that allow growth of single *lacpB* deletants (results not shown). Moreover, glycerol-grown double mutant mycelia transferred to lactose-AMM2 were unable to form new biomass even after a prolonged fermentation time and, unsurprisingly, lactose consumption could not be detected (Fig. 5). The specific lactose-uptake rates – assessed with the same means as in the single deletion and retransformant mutants – did not reflect any statistically relevant uptake by the double *lacp* permease mutant (Table 1).

The expression profile of *lacpB* is different from that of *lacpA*

To investigate the regulation of *lacpB*, its expression profile was addressed at the transcript level. As shown in Fig. 6(a), *lacpB* in the wild-type strain was strongly induced in the presence of lactose as early as 3 h after medium transfer of glycerol-grown mycelia. In the presence of D-galactose, a modest response was evident at 3 h, and no response could be observed at the later time points (6 and 12 h).

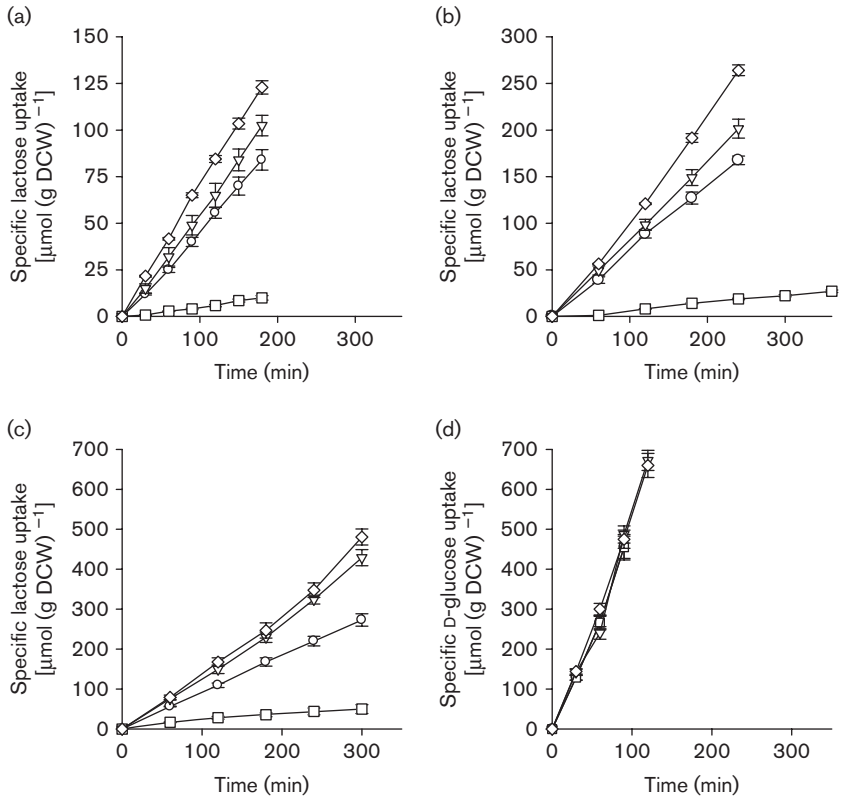

Fig. 4. (a, b, c) Specific lactose uptake in the wild-type (circles) and a *lacpB* deletant (squares), as well as the 2-copy (down-pointed triangles) and 5-copy (diamonds) *lacpB* multicopy strains in media initially containing 0.2 mM (a), 0.5 mM (b) or 2 mM (c) lactose. (d) Specific D-glucose uptake in the same strains in a medium initially containing 2 mM D-glucose. Note that the *y*-axes are differently scaled in (a), (b), (c) and (d) due to the different initial substrate concentration in each experiment. As a consequence, one cannot visually compare the apparent uptake rates between different panels.

Table 1. Lactose uptake in wild-type *A. nidulans* and in *lacpB* deletant, *lacpB* multicopy and *lacpA*/*lacpB* double mutant strains at different substrate concentrations

Differences between data from the monocopy retransformant and the wild-type control R21 were statistically irrelevant ($P<0.1$ %).

A. nidulans strain	Lactose concentration (mM)	Specific uptake rate [µmol lactose min^{-1} (g DCW)$^{-1}$]
Wild-type (R21)	0.2	0.523 ± 0.05
	0.5	0.702 ± 0.07
	2.0	0.893 ± 0.08
Δ*lacpB*	0.2	0.075 ± 0.01
	0.5	0.077 ± 0.01
	2.0	0.196 ± 0.03
AOEF011.9 (two *lacpB* copies)	0.2	0.510 ± 0.06
	0.5	0.834 ± 0.07
	2.0	1.411 ± 0.12
AOEF011.7 (five *lacpB* copies)	0.2	0.651 ± 0.05
	0.5	1.067 ± 0.12
	2.0	1.468 ± 0.14
Δ*lacpA*/Δ*lacpB*	0.2	<0.05
	0.5	<0.05
	2.0	<0.05

In contrast, *lacpA* responded to the monosaccharide D-galactose at least as strongly as to lactose at 3 h, although later on, the transcript levels on D-galactose faded (Fig. 6b). Another difference between the two *lacp* genes was observed in the presence of L-arabinose: as reported previously, L-arabinose provokes induction of *lacpA* (Fekete *et al.*, 2012), but *lacpB* expression could not be observed. As could be expected, the strongly repressing monosaccharide D-glucose did not provoke transcript accumulation from either *lacp* gene at any of the three time points, and this was also the case for the pentose D-xylose and the D-glucose C-2 epimer D-mannose (Fig. 6a, b).

In the *Aspergillus* genome database (AspGD; http://www.aspergillusgenome.org), *lacpB* (AN02814) was auto-annotated as a putative cellobiose (4-*O*-*β*-D-glucopyranosyl-D-glucose) transporter gene (Cerqueira *et al.*, 2014), as it encodes the structural orthologue of the *Neurospora crassa* CDT-1 transporter (locus NCU00801), a permease that was shown to transport cellobiose when expressed in yeast (Galazka *et al.*, 2010). We tested the physiological response of both *A. nidulans lacp* genes to the presence of cellobiose in the growth medium. As Fig. 6(a, b) shows, *lacpB* was strongly induced in the wild-type strain

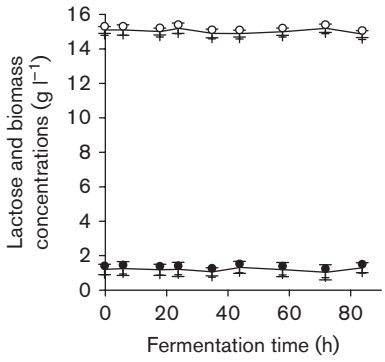

Fig. 5. Time profile of growth (filled symbols) as well as residual lactose concentration (empty symbols) in a batch fermentation of an *A. nidulans lacpA*/*lacpB* double deletion mutant (female symbols) in media initially containing 15 g lactose l^{-1}. Glycerol-germinated pregrown mycelia were used as the inoculum. Identical results were obtained for other independently obtained double lactose permease mutants.

particularly at 3 and 6 h, while no induction could be observed for *lacpA* using Northern blot transcript analysis.

Cellobiose is a low molecular mass inducer of fungal cellulolytic systems (for a review, see Seiboth *et al.*, 2007). To verify whether its inducing effect on *lacpB* transcription is related to lignocellulose breakdown, we monitored the responses of the two *lacp* genes in the presence of another β-linked glucopyranose dimer, sophorose (2-*O*-β-D-glucopyranosyl-D-glucose), a potent cellulolytic inducer in *Trichoderma reesei* but not in *Aspergillus niger* (Sternberg & Mandels, 1980; Gielkens *et al.*, 1999). As shown in Fig. 6(c), the induction response to sophorose was very similar to that of cellobiose, i.e. no transcript could be detected for *lacpA*, while *lacpB* was strongly induced, particularly at 3 h and 6 h after medium shift.

The two differentially expressed lactose permeases appear to be jointly responsible for lactose uptake in *A. nidulans* under physiological conditions (see above). To probe whether their expression is coordinated, we assessed how one lactose permease gene reacts to the loss of the other

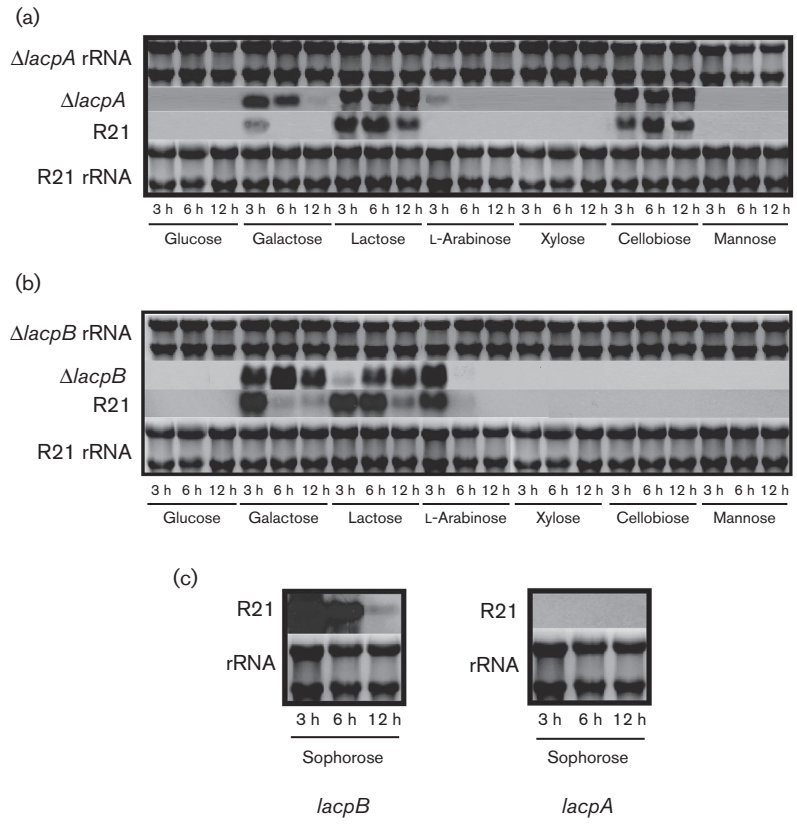

Fig. 6. (a, b) Transcript analysis of the *A. nidulans lacpB* (a) and *lacpA* (b) gene in wild-type- and single *lacp*-negative backgrounds. rRNA (28S and 18S) was visualized with ethidium bromide and is shown in the top and bottom panels as a control for RNA sample quality and quantity. (c) Expression of the *A. nidulans lacpB* (left) and *lacpA* (right) genes in response to sophorose.

in single permease deleted backgrounds (i.e. either Δ*lacpA* or Δ*lacpB*) (Fig. 6a, b). *lacpB* appeared better induced on D-galactose while its response seemed prolonged on lactose and, interestingly, on cellobiose in the *lacpA* deletion strain. Similarly, the *lacpA* response to D-galactose was seemingly extended in the absence of *lacpB*, although its expression in the presence of lactose appeared to be delayed. *lacpA* remained irresponsive to cellobiose in the *lacpB* deletant. These observations point towards possible interplay between the lactose permeases and/or their regulatory circuits.

LacpB may be a common component of lactose and cellulose catabolism in *A. nidulans*

The finding that *lacpB* is strongly induced by cellobiose led us to investigate whether the respective knockout strains exhibit a growth phenotype on this disaccharide, when serving as the sole carbon source. Controlled fermentations were performed to obtain kinetic profiles for biomass formation and cellobiose consumption. As shown in Fig. 7, the *lacpA* and *lacpB* single mutants, as well as the double *lacpA/lacpB* mutant, displayed a clear cellobiose phenotype relative to the wild-type reference and the monocopy retransformant strain. (NB these latter two strains again behaved essentially the same.) In a *lacpA*-negative background, cellobiose was consumed more rapidly ($P<0.1$ %) (Fig. 7a), and consequently, fungal biomass formation was significantly ($P<1$ %) faster in comparison to the control (Fig. 7b). This correlates with the *lacpB* expression profile observed in this mutant (see above). On the contrary, the growth rate and cellobiose consumption of the *lacpB* deletant and the double permease mutant strains were significantly ($P<1$ %) lower. These results collaborate with our transcript analyses and demonstrate that the product of *lacpB* is involved in the transport of cellobiose in *A. nidulans*; LacpB should, therefore, be considered an important component of the cellulolytic system of this fungus. It must be noted, however, that growth of the *lacpB* deletion mutants on cellobiose was reduced, but not eliminated: each of our permease mutants germinated, grew and sporulated on this disaccharide. These results strongly suggested that cellobiose uptake in *A. nidulans* prominently involves additional, hitherto unknown, transport systems.

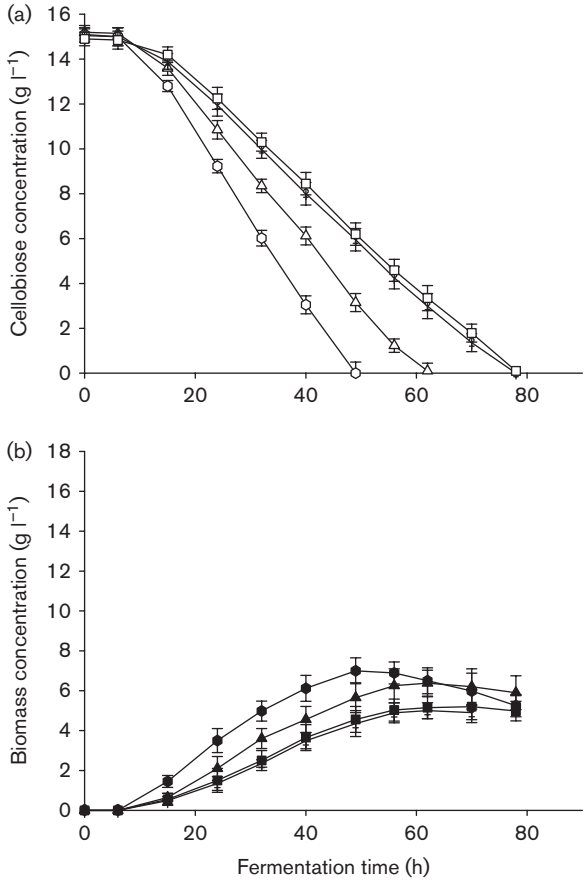

Fig. 7. Time profiles of residual cellobiose concentration (a) and growth (b) in batch fermentations of the monocopy *lacpB* retransformant (up-pointed triangles), the *lacpA* deletant (hexagons), the *lacpB* deletant (squares) and the *lacpA*/*lacpB* double permease deleted (female symbols) *A. nidulans* strains. The monocopy retransformant essentially behaves like wild-type control strain R21. Media were inoculated with conidiospores.

DISCUSSION

As an abundant by-product of cheese manufacture, lactose has long been used as a growth substrate for the production of fungal metabolites such as penicillin and hydrolytic enzymes (Roelfsema *et al.*, 2010). However, from several perspectives, it is an unusual disaccharide. It exclusively occurs in mammalian milk where it makes up 2–8 % of the dry weight. Saprophytic fungi and fungal plant, entomopathogens and mycopathogens do not encounter lactose in their natural habitats and thus – in the absence of tailor-made enzymes and transporters – they either are unable to utilize it or do so at a low rate. That said, the sugar transport potential of the soil-borne saprophyte *A. nidulans*, whose genome is predicted to harbour over 400 genes encoding putative MFS 12-transmembrane-domain proteins (Wortman *et al.*, 2009), appears diverse enough for some uptake of lactose, enabling its subsequent intracellular hydrolysis into D-glucose and D-galactose, which are both natural carbon sources for most fungi. Indeed, *Aspergillus* versatility is handsomely illustrated by the variation of occurence of LacpA and the seven MFS proteins structurally most similar to it (documented in Fig. S1).

In this work, we demonstrated that *A. nidulans* has two permeases, LacpA and LacpB, that transport the gratuitous carbon source lactose under standard laboratory conditions (i.e. concentrations up to 2 %, w/v). We cannot exclude the existence of other permeases that have the biochemical

capability to transport lactose over the plasma membrane. However, the expression of such transporter(s) is apparently not able to initiate germination and/or enable sustained growth on lactose in the absence of both LacpA and LacpB.

In general, conidiospore germination efficiency decreases with the increasing osmolarity of the medium (for a quantitative analysis in *A. niger*, see Wucherpfennig *et al.*, 2011). Lactose is amongst the least osmotically active sugars (Mustapha *et al.*, 1997). Despite this fact, we found that wild-type *A. nidulans* could not germinate at or over 5 % (w/v) lactose. This is remarkable since other aspergilli of industrial interest are routinely grown on substrates containing well over 10 % carbohydrate (e.g. Karaffa *et al.*, 2015).

Based on the residual lactose uptake of the respective single permease deletants, LacpB may have a significant function during germination and early growth on lactose, while LacpA appears accessory. However, only deletion of both *lacp* genes resulted in a complete cessation of growth on lactose. This is in contrast to the situation in the lactose fermenting yeast *K. lactis*, which expresses only one physiological lactose transporter, Lac12 (Riley *et al.*, 1987; Lodi & Donnini, 2005). Both LacpA and LacpB are structurally related to Lac12 (for the sequence, see Chang & Dickson, 1988) as these proteins share far more similarity than expected from the mere existence of 12 transmembranal domains in each of these functional homologues. However, the sequences of LacpA and LacpB are more similar to each other than either of them is to Lac12 (Fekete *et al.*, 2012).

The difference in the response to cellobiose, as well as to its positional isomer sophorose, was absolute, as we found that *lacpB* transcripts were produced abundantly upon induction by these two β-linked glucopyranose dimers, while we could not observe any expression of *lacpA*. Cellobiose and lactose are structurally similar epimers only differing at the C4 position of the nonreducing monosaccharide unit, and our results suggest that gratuitous lactose catabolism is intimately connected to the fungal cellulolytic system. This functional link has previously been described for *T. reesei*, for which lactose is the only soluble carbon source that induces cellulase and hemicellulase enzymes in economically viable industrial conditions (Bischof *et al.*, 2013). The lactose transporter Crt1 (annotated protein identifier Trire2 : 3405) appears to be essential for growth on lactose and its deletion prevents lactose-induced cellulase gene expression (Ivanova *et al.*, 2013; Zhang *et al.*, 2013). The structural LacpB orthologue from *N. crassa* (locus NCU00801) has been described as a potent cellobiose transporter (Galazka *et al.*, 2010). In contrast, the *A. niger* genome does not specify orthologues for *lacpB* and *lacpA*, and interestingly, sophorose does not induce cellulolytic genes in this species (Gielkens *et al.*, 1999). Furthermore, this *Aspergillus* species is known to hydrolyse lactose exclusively extracellular with GH35 glycoside hydrolases (see, for example: Nevalainen, 1981; O'Connell & Walsh, 2010).

Our current results suggest that LacpB appears to be integral to cellulose catabolism. Meanwhile, LacpA may primarily be involved in the degradation of type I arabinogalactan, a pectic plant cell wall polymer. *A. nidulans* produces arabinogalactan endo-β-1,4-galactanase (EC 3.2.1.89; locus identifier AN5727), an extracellular GH53 that ultimately yields D-galactose and β-1,4-galactobiose (Michalak *et al.*, 2012; Otten *et al.*, 2013). We hypothesize that LacpA is a permease for β-1,4-galactobiose that facilitates its intracellular hydrolysis by the GH2 β-1,4-galactosidase BgaD and may transport lactose as a side activity, as these two disaccharides differ at the C4 of the reducing monosaccharide unit only. In support of this thesis, we had previously shown that D-galactose – the monomeric end product of extracellular endo-β-1,4-galactanase activity – is a potent inducer of the clustered *lacpA* and *bgaD* genes in *A. nidulans*, and that two putative extracellular GH35 β-1,4-galactosidase genes are not expressed in this fungus under the tested conditions (Fekete *et al.*, 2012). In conclusion, the two-pronged uptake system of lactose in the soil-borne saprophyte *A. nidulans* may represent an unexpected interface of two important hydrolytic systems necessary to degrade plant cell walls and to use them as growth substrate.

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REFERENCES

Aghcheh, R. K. & Kubicek, C. P. (2015). Epigenetics as an emerging tool for improvement of fungal strains used in biotechnology. *Appl Microbiol Biotechnol* **99**, 6167–6181.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Anisimova, M. & Gascuel, O. (2006). Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst Biol* **55**, 539–552.

Baruffini, E., Goffrini, P., Donnini, C. & Lodi, T. (2006). Galactose transport in *Kluyveromyces lactis*: major role of the glucose permease Hgt1. *FEMS Yeast Res* **6**, 1235–1242.

Bischof, R., Fourtis, L., Limbeck, A., Gamauf, C., Seiboth, B. & Kubicek, C. P. (2013). Comparative analysis of the *Trichoderma reesei* transcriptome during growth on the cellulase inducing substrates wheat straw and lactose. *Biotechnol Biofuels* **6**, 127.

Cardinali, G., Vollenbroich, V., Jeon, M. S., de Graaf, A. A. & Hollenberg, C. P. (1997). Constitutive expression in *gal7* mutants of *Kluyveromyces lactis* is due to internal production of galactose as an inducer of the Gal/Lac regulon. *Mol Cell Biol* **17**, 1722–1730.

Cerqueira, G. C., Arnaud, M. B., Inglis, D. O., Skrzypek, M. S., Binkley, G., Simison, M., Miyasato, S. R., Binkley, J., Orvis, J. & other authors (2014). The *Aspergillus* Genome Database: multispecies curation and

incorporation of RNA-Seq data to improve structural gene annotations. *Nucleic Acids Res* **42**, D705–D710.

Chang, Y. -D. & Dickson, R. C. (1988). Primary structure of the lactose permease gene from the yeast *Kluyveromyces lactis*. Presence of an unusual transcript structure. *J Biol Chem* **263**, 16696–16703.

Clutterbuck, A. J. (1974). *Aspergillus nidulans*. In *Handbook of Genetics, Bacteria, Bacteriophages, and Fungi*, vol. 1, pp. 447–510. Edited by R. C. King. New York: Plenum.

Coghill, R. D. & Moyer, A. J. (1947). Method for production of increased yields of penicillin. US Patent 2,423,873.

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

Diniz, R. H. S., Silveira, W. B., Fietto, L. G. & Passos, F. M. L. (2012). The high fermentative metabolism of *Kluyveromyces marxianus* UFV-3 relies on the increased expression of key lactose metabolic enzymes. *Antonie van Leeuwenhoek* **101**, 541–550.

Fekete, E., Karaffa, L., Sándor, E., Seiboth, B., Biró, S., Szentirmai, A. & Kubicek, C. P. (2002). Regulation of formation of the intracellular β-galactosidase activity of *Aspergillus nidulans*. *Arch Microbiol* **179**, 7–14.

Fekete, E., Karaffa, L., Seiboth, B., Fekete, É., Kubicek, C. P. & Flipphi, M. (2012). Identification of a permease gene involved in lactose utilisation in *Aspergillus nidulans*. *Fungal Genet Biol* **49**, 415–425.

Galazka, J. M., Tian, C., Beeson, W. T., Martinez, B., Glass, N. L. & Cate, J. H. (2010). Cellodextrin transport in yeast for improved biofuel production. *Science* **330**, 84–86.

Gänzle, M. G., Haase, G. & Jelen, P. (2008). Lactose: crystallization, hydrolysis and value-added derivatives. *Int Dairy J* **18**, 685–694.

Gielkens, M. M., Dekkers, E., Visser, J. & de Graaff, L. H. (1999). Two cellobiohydrolase-encoding genes from *Aspergillus niger* require D-xylose and the xylanolytic transcriptional activator XlnR for their expression. *Appl Environ Microbiol* **65**, 4340–4345.

Gödecke, A., Zachariae, W., Arvanitidis, A. & Breunig, K. D. (1991). Coregulation of the *Kluyveromyces lactis* lactose permease and β-galactosidase genes is achieved by interaction of multiple LAC9 binding sites in a 2.6 kbp divergent promoter. *Nucleic Acids Res* **19**, 5351–5358.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* **59**, 307–321.

Ivanova, C., Bååth, J. A., Seiboth, B. & Kubicek, C. P. (2013). Systems analysis of lactose metabolism in *Trichoderma reesei* identifies a lactose permease that is essential for cellulase induction. *PLoS One* **8**, e62631.

Jónás, Á., Fekete, E., Flipphi, M., Sándor, E., Jäger, S., Molnár, Á. P., Szentirmai, A. & Karaffa, L. (2014). Extra- and intracellular lactose catabolism in *Penicillium chrysogenum*: phylogenetic and expression analysis of the putative permease and hydrolase genes. *J Antibiot (Tokyo)* **67**, 489–497.

Karaffa, L., Coulier, L., Fekete, E., Overkamp, K. M., Druzhinina, I. S., Mikus, M., Seiboth, B., Novák, L., Punt, P. J. & Kubicek, C. P. (2013). The intracellular galactoglycome in *Trichoderma reesei* during growth on lactose. *Appl Microbiol Biotechnol* **97**, 5447–5456.

Karaffa, L., Díaz, R., Papp, B., Fekete, E., Sándor, E. & Kubicek, C. P. (2015). A deficiency of manganese ions in the presence of high sugar concentrations is the critical parameter for achieving high yields of itaconic acid by *Aspergillus terreus*. *Appl Microbiol Biotechnol* **99**, 7937–7944.

Katoh, K. & Toh, H. (2010). Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* **26**, 1899–1900.

Kumari, S., Panesar, P. S. & Panesar, R. (2011). Production of β-galactosidase using novel yeast isolate from whey. *Int J Dairy Sci* **6**, 150–157.

Lodi, T. & Donnini, C. (2005). Lactose-induced cell death of β-galactosidase mutants in *Kluyveromyces lactis*. *FEMS Yeast Res* **5**, 727–734.

Marwaha, S. S. & Kennedy, J. F. (1988). Whey – pollution problem and potential utilization. *Int J Food Sci Technol* **23**, 323–336.

Michalak, M., Thomassen, I. V., Roytio, H., Ouwehand, A. C., Meyer, A. S. & Mikkelsen, J. D. (2012). Expression and characterization of an endo-1,4-β-galactanase from *Emericella nidulans* in *Pichia pastoris* for enzymatic design of potentially prebiotic oligosaccharides from potato galactans. *Enzyme Microb Technol* **50**, 121–129.

Mustapha, A., Jiang, T. & Savaiano, D. A. (1997). Improvement of lactose digestion by humans following ingestion of unfermented acidophilus milk: influence of bile sensitivity, lactose transport, and acid tolerance of *Lactobacillus acidophilus*. *J Dairy Sci* **80**, 1537–1545.

Nayak, T., Szewczyk, E., Oakley, C. E., Osmani, A., Ukil, L., Murray, S. L., Hynes, M. J., Osmani, S. A. & Oakley, B. R. (2006). A versatile and efficient gene-targeting system for *Aspergillus nidulans*. *Genetics* **172**, 1557–1566.

Nevalainen, K. M. H. (1981). Induction, isolation, and characterization of *Aspergillus niger* mutant strains producing elevated levels of β-galactosidase. *Appl Environ Microbiol* **41**, 593–596.

O'Connell, S. & Walsh, G. (2010). A novel acid-stable, acid-active β-galactosidase potentially suited to the alleviation of lactose intolerance. *Appl Microbiol Biotechnol* **86**, 517–524.

Otten, H., Michalak, M., Mikkelsen, J. D. & Larsen, S. (2013). The binding of zinc ions to *Emericella nidulans* endo-β-1,4-galactanase is essential for crystal formation. *Acta Cryst* **F69**, 850–854.

Panesar, P. S. & Kennedy, J. F. (2012). Biotechnological approaches for the value addition of whey. *Crit Rev Biotechnol* **32**, 327–348.

Panesar, P. S., Panesar, R., Singh, R. S., Kennedy, J. F. & Kumar, H. (2006). Microbial production, immobilization and applications of β-D-galactosidase. *J Chem Technol Biotechnol* **81**, 530–543.

Rigamonte, T. A., Silveira, W. B., Fietto, L. G., Castro, I. M., Breunig, K. D. & Passos, F. M. L. (2011). Restricted sugar uptake by sugar-induced internalization of the yeast lactose/galactose permease Lac12. *FEMS Yeast Res* **11**, 243–251.

Riley, M. I., Sreekrishna, K., Bhairi, S. & Dickson, R. C. (1987). Isolation and characterization of mutants of *Kluyveromyces lactis* defective in lactose transport. *Mol Gen Genet* **208**, 145–151.

Roelfsema, W. A., Kuster, B. F. M., Heslinga, M. C., Pluim, H. & Verhage, M. (2010). *Lactose and Derivatives. Ullmann's Encyclopedia of Industrial Chemistry*, 7th edn. New York: Wiley.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Seiboth, B., Pakdaman, B. S., Hartl, L. & Kubicek, C. P. (2007). Lactose metabolism in filamentous fungi: how to deal with an unknown substrate. *Fungal Biol Rev* **21**, 42–48.

Silva, M. F., Fornari, R. C. G., Mazutti, M. A., de Oliveira, D., Padilha, F. F., Cichoski, A. J., Cansian, R. L., Di Luccio, M. & Treichel, H. (2009). Production and characterization of xantham gum by *Xanthomonas campestris* using cheese whey as sole carbon source. *J Food Eng* **90**, 119–123.

Sternberg, D. & Mandels, G. R. (1980). Regulation of the cellulolytic system in *Trichoderma reesei* by sophorose: induction of cellulase and repression of β-glucosidase. *J Bacteriol* **144**, 1197–1199.

Tilburn, J., Scazzocchio, C., Taylor, G. G., Zabicky-Zissman, J. H., Lockington, R. A. & Davies, R. W. (1983). Transformation by integration in *Aspergillus nidulans*. *Gene* **26**, 205–221.

Wortman, J. R., Gilsenan, J. M., Joardar, V., Deegan, J., Clutterbuck, J., Andersen, M. R., Archer, D., Bencina, M., Braus, G. & other authors (2009). The 2008 update of the *Aspergillus nidulans* genome annotation: a community effort. *Fungal Genet Biol* **46** (Suppl. 1), S2–S13.

Wucherpfennig, T., Hestler, T. & Krull, R. (2011). Morphology engineering–osmolality and its effect on *Aspergillus niger* morphology and productivity. *Microb Cell Fact* **10**, 58.

Yu, J. H., Hamari, Z., Han, K. H., Seo, J. A., Reyes-Domínguez, Y. & Scazzocchio, C. (2004). Double-joint PCR: a PCR-based molecular tool for gene manipulations in filamentous fungi. *Fungal Genet Biol* **41**, 973–981.

Zhang, W., Kou, Y., Xu, J., Cao, Y., Zhao, G., Shao, J., Wang, H., Wang, Z., Bao, X. & other authors (2013). Two major facilitator superfamily sugar transporters from *Trichoderma reesei* and their roles in induction of cellulase biosynthesis. *J Biol Chem* **288**, 32861–32872.

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