

Recombinant *Aspergillus* β -galactosidases as a robust glycomic and biotechnological tool

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Supplemental Tables

Supplemental Table S1. Influence of galactose and glucose on enzymatic activity.

Activity of recombinant *Aspergillus* galactosidases and non-recombinant *A. oryzae* galactosidase (*) in the presence of different concentrations of galactose and glucose. For each enzyme, the activities at various conditions are shown in relation to the maximal activity measured for that enzyme. Average values (% activity) +/- standard error are shown.

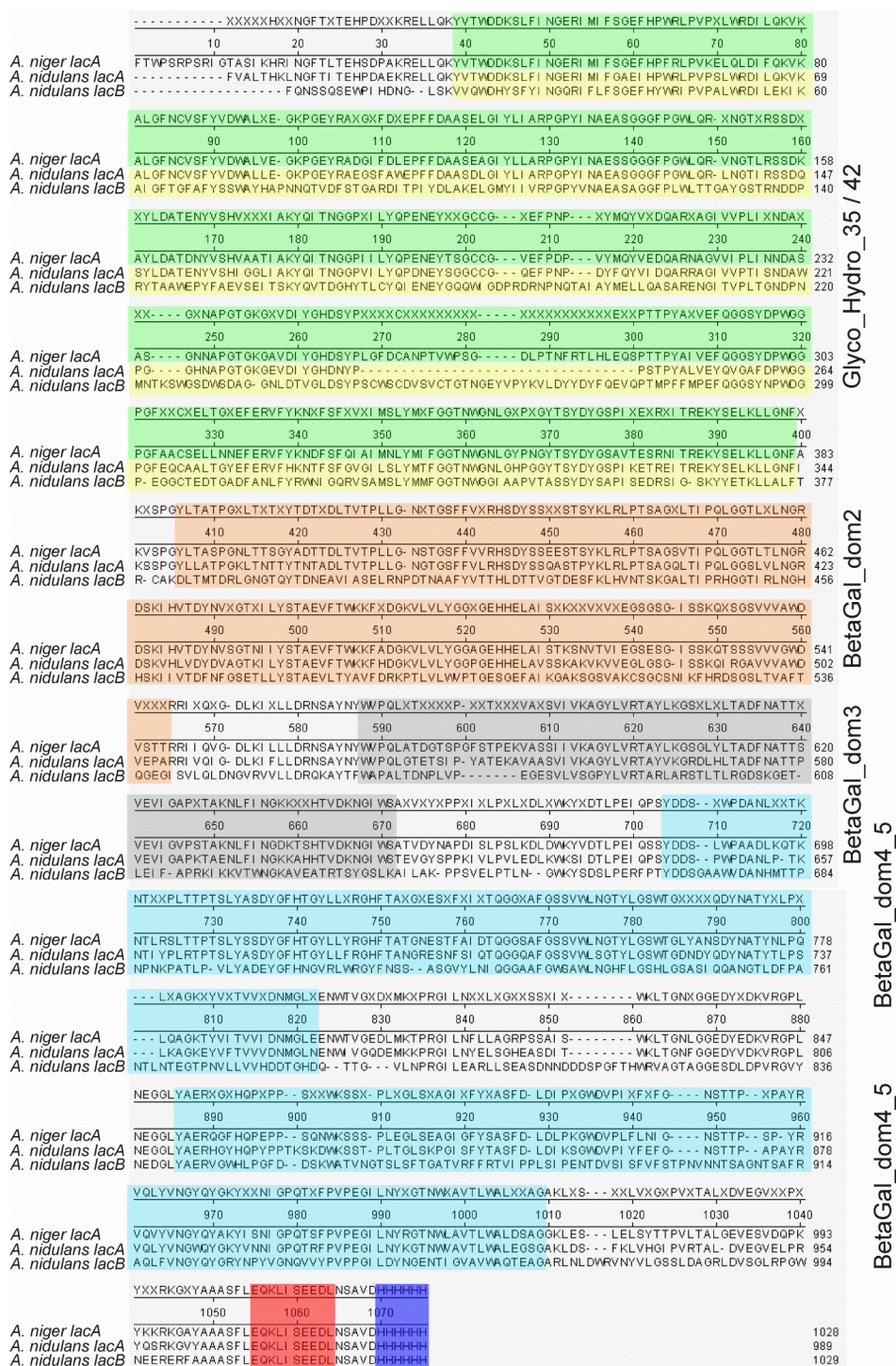
galactose [mM]	<i>A. niger</i> rIacA	<i>A. nidulans</i> rIacA	<i>A. nidulans</i> rIacB	<i>A.oryzae</i>*
0	<u>100 +/- 0.0</u>	58.9 +/- 0.1	<u>100 +/- 0.5</u>	<u>100 +/- 2.6</u>
0.95	90.3 +/- 1.5	<u>100 +/- 0.1</u>	71.9 +/- 4.2	30.4 +/- 5.4
4.75	65.8 +/- 1.7	92.2 +/- 3.4	33.7 +/- 2.7	23.8 +/- 1.2
9.5	47.9 +/- 0.3	39.7 +/- 4.8	24.8 +/- 2.7	23.4 +/- 4.6
47.5	15.9 +/- 0.2	34.6 +/- 5.9	4.6 +/- 0.7	12.5 +/- 3.0
95	5.1 +/- 0.2	16.6 +/- 0.2	2.6 +/- 0.3	8.9 +/- 1.7
475	2.5 +/- 0.1	4.14 +/- 0.0	0.2 +/- 0.1	2.5 +/- 1.4
950	1.0 +/- 0.3	1.72 +/- 0.3	0.2 +/- 0.5	0.3 +/- 0.0
glucose [mM]	<i>A. niger</i> rIacA	<i>A. nidulans</i> rIacA	<i>A. nidulans</i> rIacB	<i>A.oryzae</i>*
0	82.2 +/- 7.3	45.3 +/- 1.7	79.6 +/- 5.9	62.6 +/- 5.3
9.5	90.2 +/- 5.2	51.7 +/- 1.0	80.1 +/- 0.3	67.5 +/- 2.9
23.75	96.5 +/- 5.3	51.2 +/- 3.4	97.1 +/- 10.4	70.0 +/- 5.5
47.5	91.6 +/- 3.1	57.3 +/- 1.8	97.7 +/- 3.1	77.7 +/- 1.5
95	99.2 +/- 0.5	71.4 +/- 3.4	99.1 +/- 13.3	86.9 +/- 5.1
190	<u>100.0 +/- 1.3</u>	93.0 +/- 2.8	<u>100.0 +/- 8.5</u>	<u>100.0 +/- 10.1</u>
950	90.8 +/- 3.2	<u>100 +/- 6.6</u>	82.2 +/- 8.2	88.1 +/- 13.0
1900	61.8 +/- 0.9	76.0 +/- 3.1	61.8 +/- 1.6	72.2 +/- 1.9

Supplemental Table S2. β -1,3 activity using lacto-*N*-tetraose as substrate.

Ratio of β -1,3 activity and β -1,4 activity after 2, 24 and 48h is shown. For *A. niger* and *A. nidulans* *rlacA*, values after 24 and 48h (marked with an asterisk) overestimate the activity as almost complete conversion of lacto-*N*-neotetraose (β -1,4 substrate) already occurred after 2h (see Figure 4 in the main manuscript).

Ratio of β-1,3 activity vs β-1,4 activity after [h] of incubation	2	24	48
<i>A. niger</i> <i>rlacA</i>	0.027	0.242*	0.616*
<i>A. nidulans</i> <i>rlacA</i>	0.043	0.429*	0.979*
<i>A. nidulans</i> <i>rlacB</i>	0.005	0.195	0.280
<i>A. oryzae</i> galactosidase	0.019	0.081	0.429

Supplemental Figures



Supplemental Fig. S1 Sequence alignment of the three recombinant galactosidases

The sequences used in the current study are depicted. Conserved sequence motifs (yellow, green, orange, grey, light blue) and C-terminal myc (red) and HIS tags (dark blue) are highlighted.

A *A. niger* rIacA

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1  FTWPSRPSRI GTASIKHRIN GFTLTEHSDP AKRELLQKQV TWDDKSLFIN GERIMIFSGE FHPFRLPVKE LQLDIFQKVK
81  ALGFNCVSYF VDWALVEGKP GEYRADGIFD LEFFFDAASE AGIYLLARPG PYINAESSGG GPPGWLQKRVN GTLRSDSKAY
161  LDATDNYVSH VAATIAKYQI TNGGPILLYQ PENEYTSGCC GVEFPDPVYM QYVEDQARNA GVVIPLINND ASASGNHAPG
241  TGKGAVDIYG HDSYPLGFC ANPTVWPSGD LPTNFRTLHL EQSPTTPYAI VEFQGGSYDP WGGPGFAACS ELLNNEFERV
321  FYNDFSFQI AIMNLYMIFG GTNWNGLGYP NGYTSYDYG AVTESRNITR EKYSELKLLG NFAKVSPGYL TASPGLNLTTS
401  GYADTTDLTV TPLLGNSTGS FVVVRHSDYS SEESTSYKLR LPTSAGSVTI PQLGGTLTLN GRDSKIHVTD YNVSGTNIIY
481  STAEVFTWKK FADGKVLVLY GGAGEHHELA ISTKSNVTVI EGSESGISSK QTSSSVVVW DVSTTRRIIQ VGDLKILLD
561  RNSAYNYWVP QLATDGTSPG FSTPEKVASS IIVKAGYLVR TAYLKGSGLY LTADFNATTS VEVIGVPSTA KNLFINGDKT
641  SHTVDKNGIW SATVDYNAPD ISLPSLKDLD WKYVDTLPEI QSSYDDSLWP AADLKQTKNT LRSLTTPTSL YSSDYGFHTG
721  YLLYRGHFTA TGNESTFAID TQGGSAFGSS VWLNGTYLGS WTGLYANSY NATYNLPQLQ AGKTYVITV IDNMGLEENN
801  TVGEDLMKTP RGINLFLLAG RPSSAISWKL TGNLGGEDYE DKVRGPLNEG GLYAERQGFH QPEPPSQNWK SSSPLEGLSE
881  AGIGFYASAF DLDLPKGDV PLFLNIGHST TSPSYRVQVY VNGYQAKYI SNIGPQTSPF VPEGILNYRG TNWLAVTLWA
961  LDSAGGKLES LELSYTTPVL TALGEVESVD QPKYKRKGA YAAASFLEQK LISEEDLNSA VDHHHHH

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B *A. nidulans* rIacA

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1  FVALTHKLNQ FTITEHPDAE KRELLQKQVYV WDDKSLFING ERIMIFGAEI HPWRLPVPSL WRDILQKKA LGFNCVSFYV
81  DWALLEKQKP EYRAEGSFAM EPFFDAASDL GIYLIARPGP YINAESGGG PPGWLQRLNG TIRSSDQSYL DATENYVSHI
161  GGLIAKYQIT NNGPVILYQP DNEYSGCCQQ QEFPNPDYFQ YVIDQARRAG IUVPTISNDA WPGGHNAPGT GKGEVDIYGH
241  DNYPPSTPYA LVEYQVQAFD PWGGPGFEQC AALTGYEFER VFHKNTFSFG VGILSLYMTF GGTNWNGLGH PGGYTSYDYG
321  SPIKETREIT REKYSELKLL GNFIKSSPGY LLATPGKLTN TTYNTADLT VTPLLGNGTG SFFVLRHSDY SSQASTPYKL
401  RLPTSAGQLT IPQLGGSLVL NGRDSKVHLV DYDVAGTKIL YSTAEVFTWK KFHDGKVLVL YGGGEHHEL AVSSKAKVKV
481  VEGLSGSISS KQIRGAVVA WVEPARIV QIGDLKIFLL DRNSAYNYWV POLGTETSIP YATEKAVAAS VIVKAGYLVR
561  TAYVKGRDLH LTADFNATTP VEVIGAPKTA ENLFINGKA HHTVDKNGIW STEVGYSPK IVLPVLEDLK WKSIDTLPEI
641  QPSYDDSPWP DANLPTKNTI YPLRTPTSLY ASDYGFHTGY LLFRGHFTAN GRESNFSIQT QGGQAFGSSV WLSGTYLGSW
721  TGDNDYQDYN ATYTLPSLKA GKEYVFTVVV DNMGLNENWI VGQDEMKKPR GILNYELSGH EASDITWKL TGNFGGEDYVD
801  KVRGPLNEGG LYAERHGYHQ PYPPTKSDW KSSTPLTGLS KPGISEFYAS FDLIDKSGWD VPIYFEFGNS TTPAPAYRVQ
881  LYVNGWYQK YVNNIGPQTR FVPEGILNY KGTNWVAVTL WALEGSGAKL DSFKLVHGIP VRTALDVEGV ELPRYQSRKG
961  VYAAASFLEQ KLISEEDLNS AVDHHHHH

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C *A. nidulans* rIacB

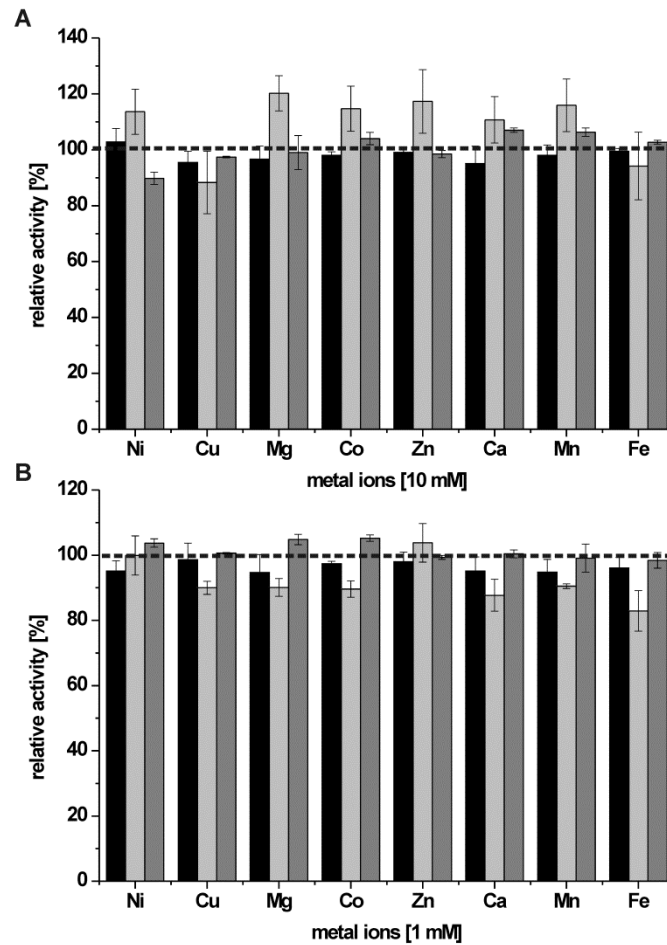
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1  FQNSSQSEWP IHDNGLSKVV QWDHYSFYIN GQRIFLFSGE FHYWRIPVPA LWRDILEKIK AIGFTGFIFY SSWAYHAPNN
81  QIVDFSTGAR DITPIYDLAK ELGMYIIVRP GPYVNAEASA GGFPLMLTTG AYGSTRNDP RYTAAWEPFY AEVSEITSKY
161  QVTDGHYTLG YQIENEYGOQ WIGDPRDRNP NOTATAYMEL LQASARENGI TVPLTGNDPN MHTKSWGSDW SDAGGNLDTV
241  GLDSYSPCWS CDVSVCCTGN GEYVYKVLVD YYDYFQEVQP TMPFFHPEFQ GGSYNPWDGP EGGCTEDTGA DFNANFYRWN
321  IGRVVSMSL YMMFGGTNWG GIAAPVTASS YDYSAPISED RSIGSKYYET KLLALFTRCA KDLTMTDRLG NGTQYTDNEA
401  VIASELNRPD TNAAFVYVTH LDTTVGTDES FKLHVNTSKG ALTIPRHGGT IRLNGHHSKI IVTDFNFGE TLLYSTAEVL
481  TYAVFDRKPT LVLWVPTGES GEFAIKGAKS GSVAKCSGCS NIKFHRDSGS LTVAFTQGEG ISVLQDNGV RVVLLDRQKA
561  YTFWAPAL TD NPLVPEGESV LVSGPYLVRT ARLARSTLTL RGDSKGETLE IFAPRKIKKV TWNGKAVEAT RTSYGLKAI
641  LAKPPSVELP TLNGWKYSDS LPERFPTVDD SGAAWVDANH MTPNPNKPA TLPVLYADEY GFHNGVRLWR GYFNSSASGV
721  YLNIQGGAAF GWSAWLNGHF LGSHLGSASI QQANGTLDFP ANTLNTEGTP NVLLVVHDDT GHDQTTGVLN PRGILEARLL
801  SEASDNDDDD SPGFTHWVA GTAGGESDLD PVRGVNEDG LYAERVGHL PGFDDSKWAT VNGTSLSFTG ATVRFRTVI
881  PPLSIPEND VSISVFSTP NVNNTSAGNT SAFRAQLFVN GYQYGRYNPY VGNQVVYPVP PGILDYNGEN TIGVAVWAQT
961  EAGARMLDW RVNYVLGSSL DAGRLDVSGL RPGWNEERER FAAAASFLEQ KLISEEDLNS AVDHHHHH

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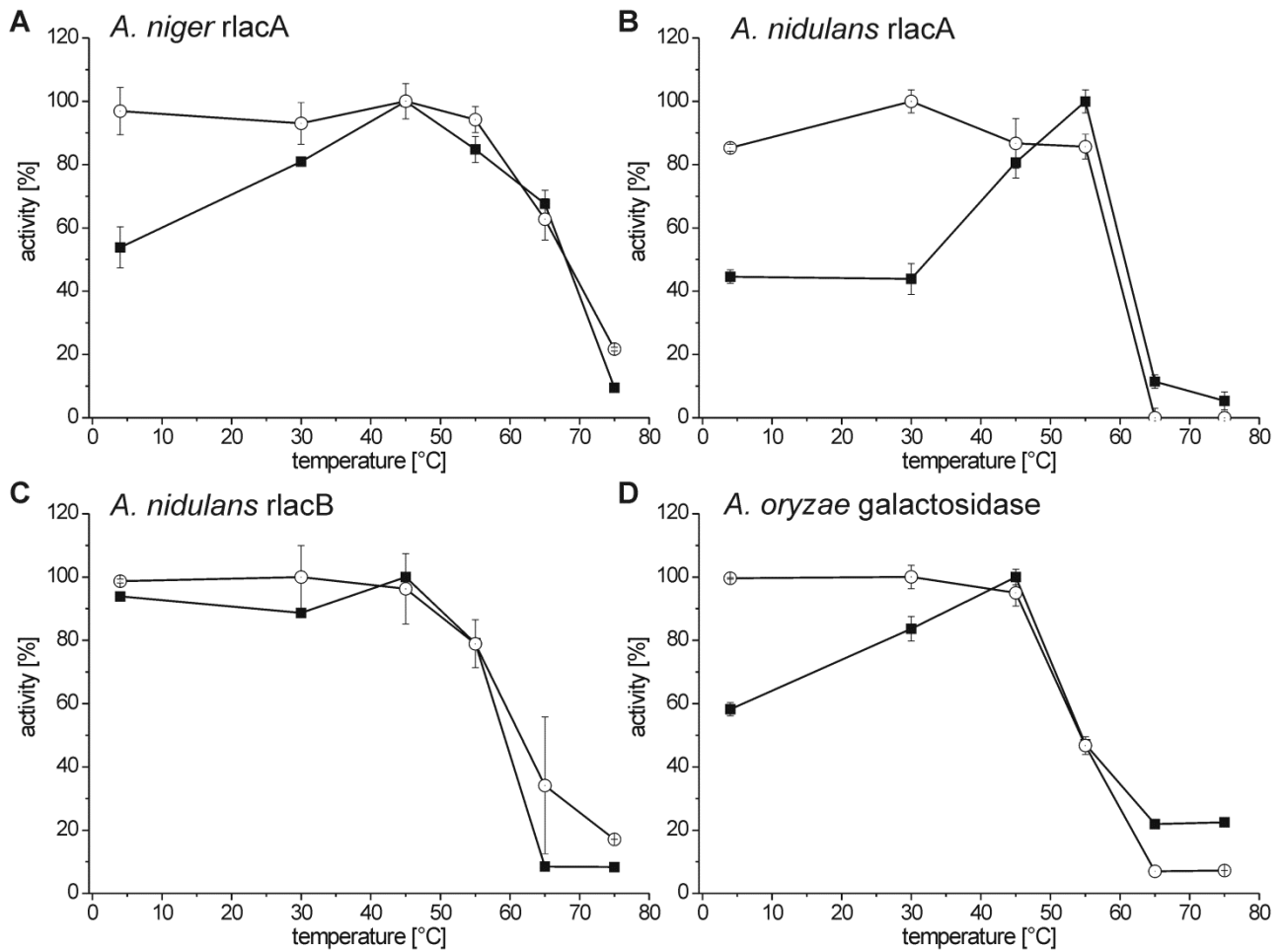
Supplemental Fig. S2 Protein identity verification by mass fingerprint analysis

Purified (A) *A. niger* rIacA (B) *A. nidulans* rIacA and (C) *A. nidulans* rIacB were subjected to digestion with trypsin and subsequently analyzed by MALDI-TOF/TOF MS. Amino acid sequences in red font represent protein sequence covered by tryptic peptides whereas underlined positions indicate trypsin cleavage sites. Sequences underlined in grey represent amino acid sequences that were attached to the native protein during cloning. Sequences highlighted in red and blue indicate recombinant c-myc and His-tag and amino acid triplets highlighted in green indicate potential N-glycosylation sites.



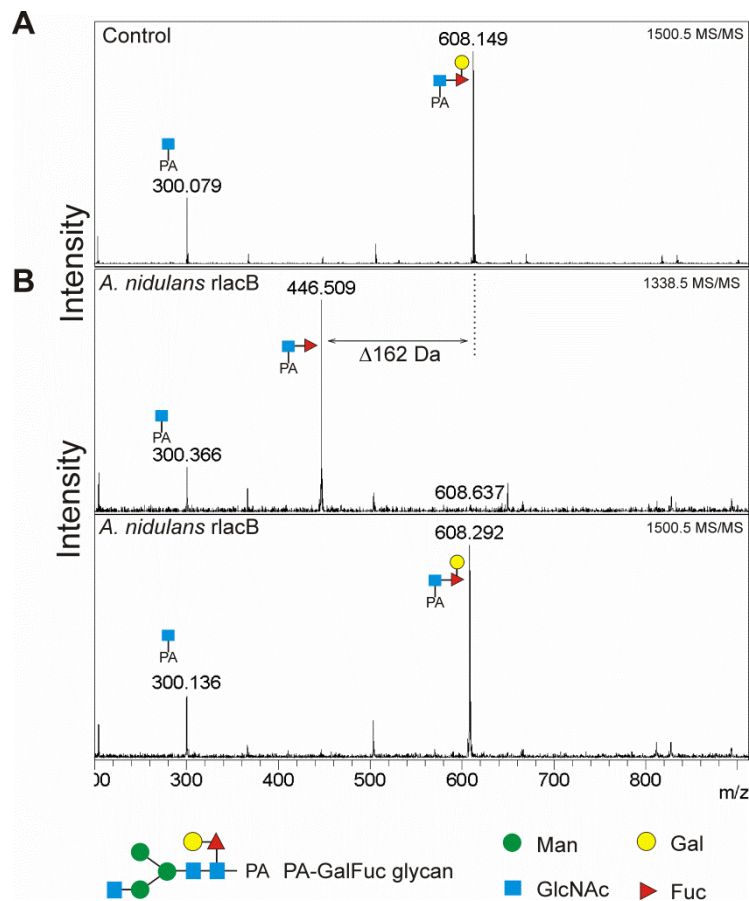
Supplemental Fig. S3 Divalent metal ion susceptibility of recombinant galactosidases.

PNPG Reactions were supplemented with (A) 10 mM and (B) 1 mM of the respective metal salts. Black bars - *A. niger* rIacA, light grey bars - *A. nidulans* rIacA and grey bars - *A. nidulans* rIacB. Values represent averages +/- standard error. % relative activity compared with standard reactions without ion supplementation (= 100 %) is shown.



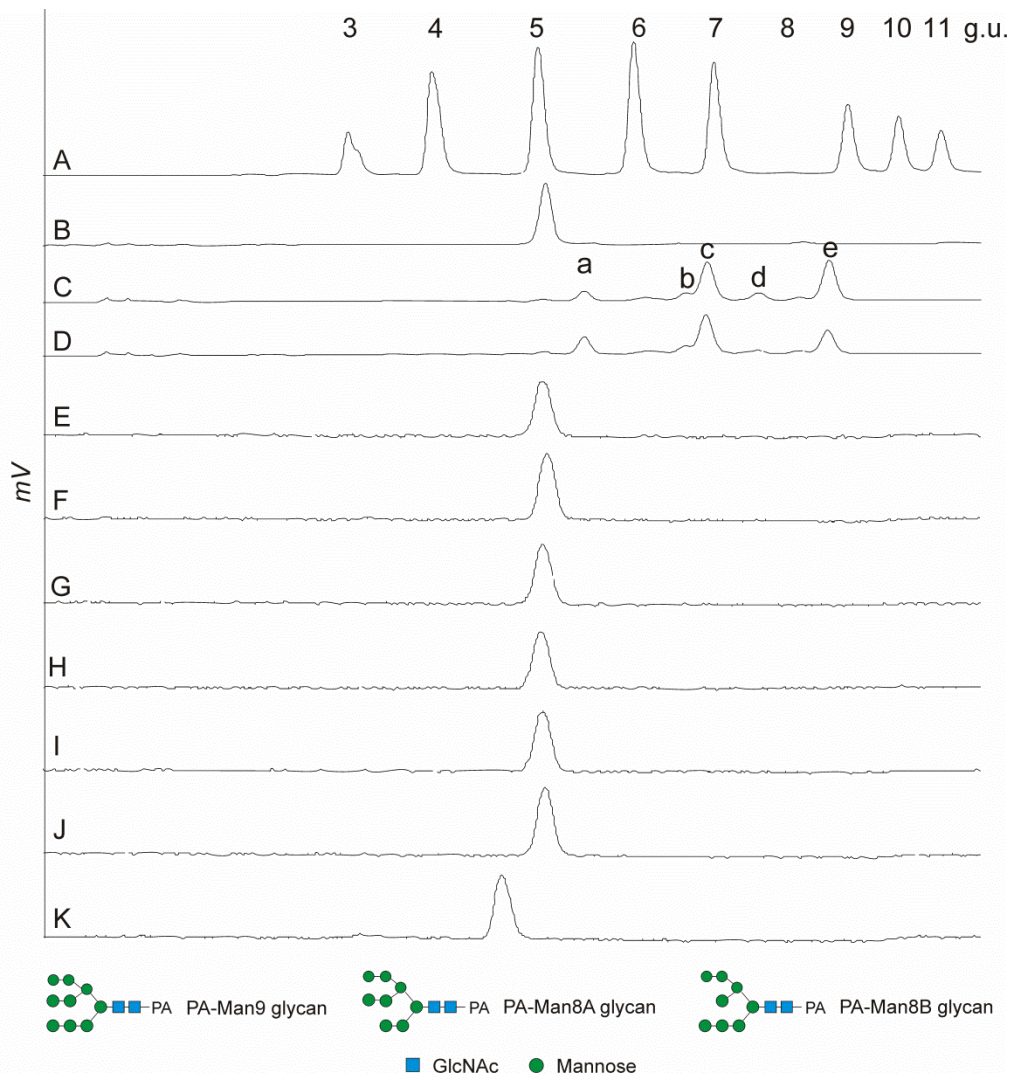
Supplemental Fig. S4 Temperature stability of *Aspergillus* β -galactosidases.

The β -galactosidases were incubated at indicated temperatures for 2 hours and remaining activity was determined by standard PNPG assay. Black squares represent incubations without BSA and open circles represent incubations supplemented with BSA. % activity \pm standard error is shown.



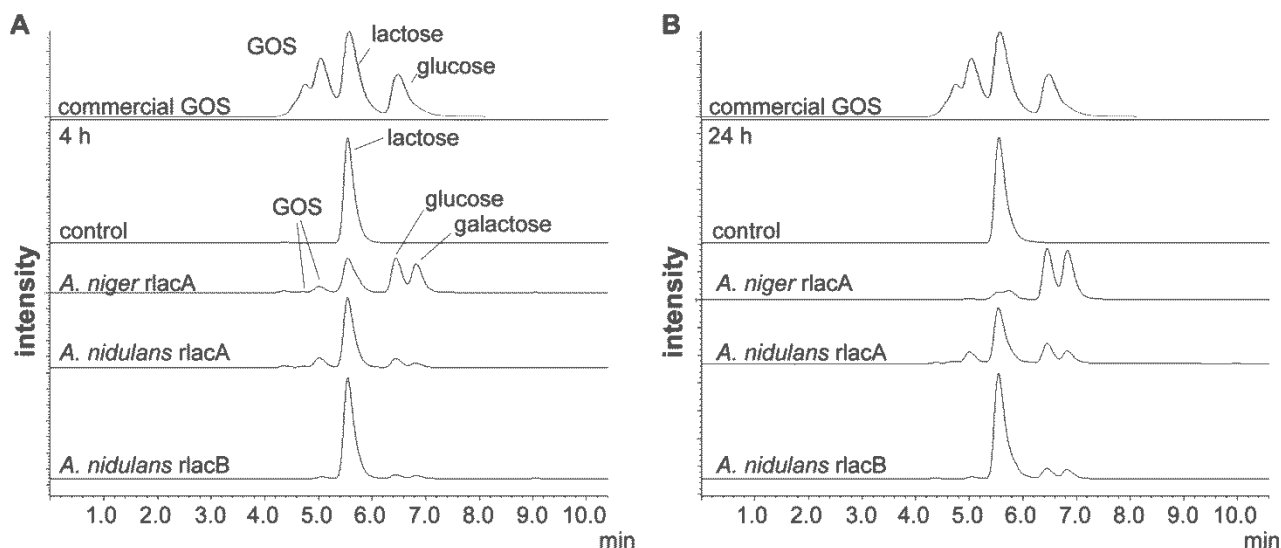
Supplemental Fig. S5 MS/MS data of PA labeled N-glycan structure containing Gal β 1,4Fuc used a substrate for recombinant β -galactosidases.

The parent ions of the major structures observed in the experiments described in Figure 5 were subject to MS/MS analyses to verify the removal of the galactose residue. (A) no enzyme control (parent ion m/z equals 1500.5 is substrate (hydrogen adduct)) (B) *A. nidulans* rIacB (parent ion m/z equals 1338.5 for processed and 1500.5 for unprocessed substrate (both as hydrogen adducts)). The data show that the 162 Da loss from the substrate is due to galactose removed from the core fucose (m/z 446 rather than 608) and not due to mannose cleavage. Comparable results were obtained for *A. niger* rIacA and *A. nidulans* rIacA (data not shown). The glycans are depicted following the nomenclature of the Consortium for Functional Glycomics (<http://www.functionalglycomics.org>).



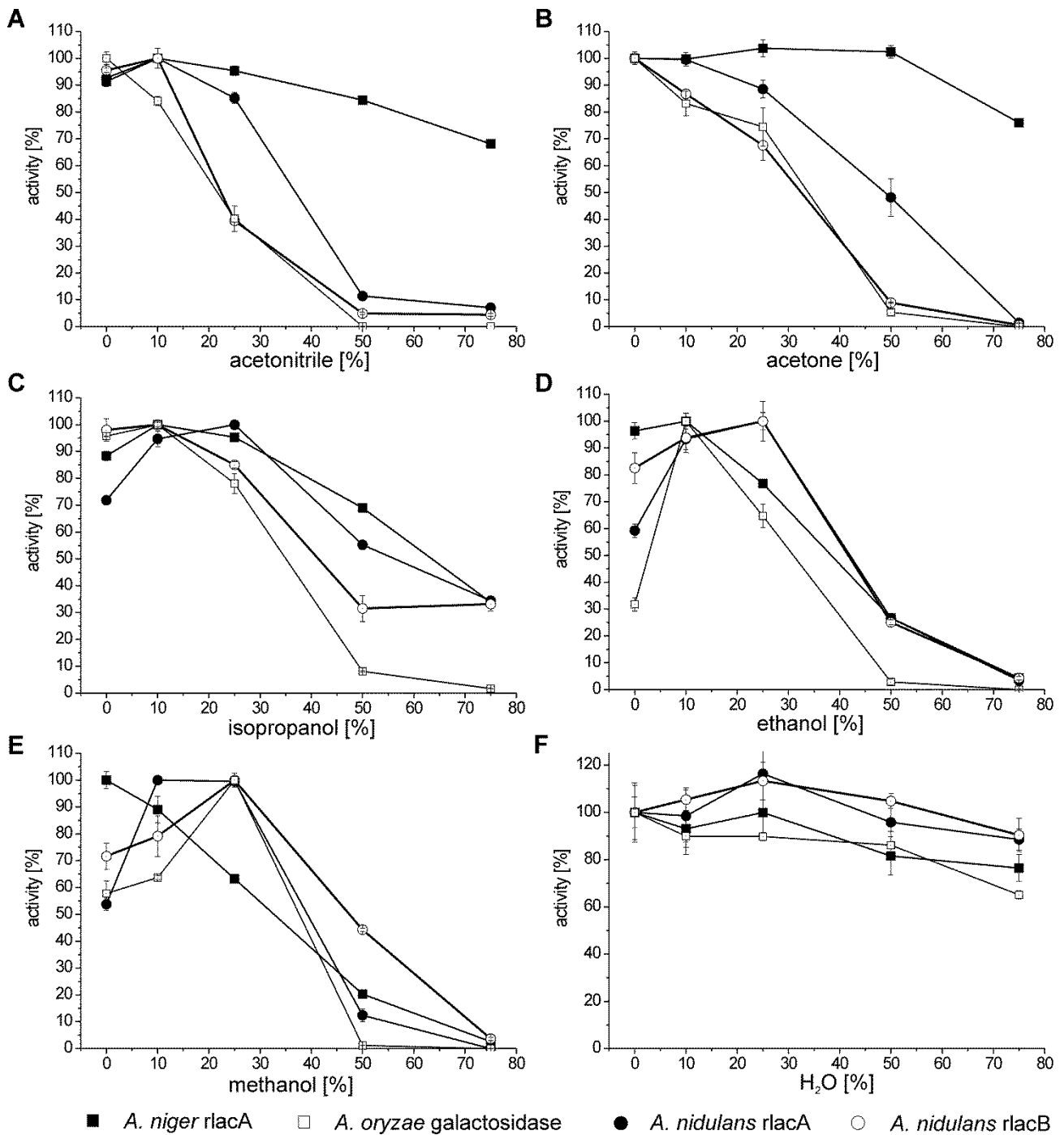
Supplemental Fig. S6 Analysis of potential mannosidase activity of the enzyme preparations.

2-aminopyridine-labeled Man9 glycan was incubated with both native and recombinant enzymes at optimal pH for fungal galactosidases (pH 4.5 or pH 5.0) and optimal pH for typical α 1,2-mannosidases (pH 6.9). (A) dextran standard (B) Man9-PA control (C) *A. oryzae* galactosidase pH 4 (D) *A. oryzae* galactosidase pH 6.9 (E) *A. niger* rlaC at pH 4.5 (F) *A. niger* rlaC at pH 6.9 (G) *A. nidulans* rlaC at pH 5.0 (H) *A. nidulans* rlaC at pH 6.9 (I) *A. nidulans* rlaB at pH 5.0 (J) *A. nidulans* rlaB at pH 6.9 and (K) positive control reaction at pH 6.9 using a recombinant mannosidase I derived from *P. pastoris* producing Man8B. Lower case letters above peaks in panel C represent (a) Man8A (b,c) Man 6 and 7 isomers (d) Man5 and (e) Man6. The glycans are depicted following the glycan nomenclature of the Consortium for Functional Glycomics (<http://www.functionalglycomics.org>).



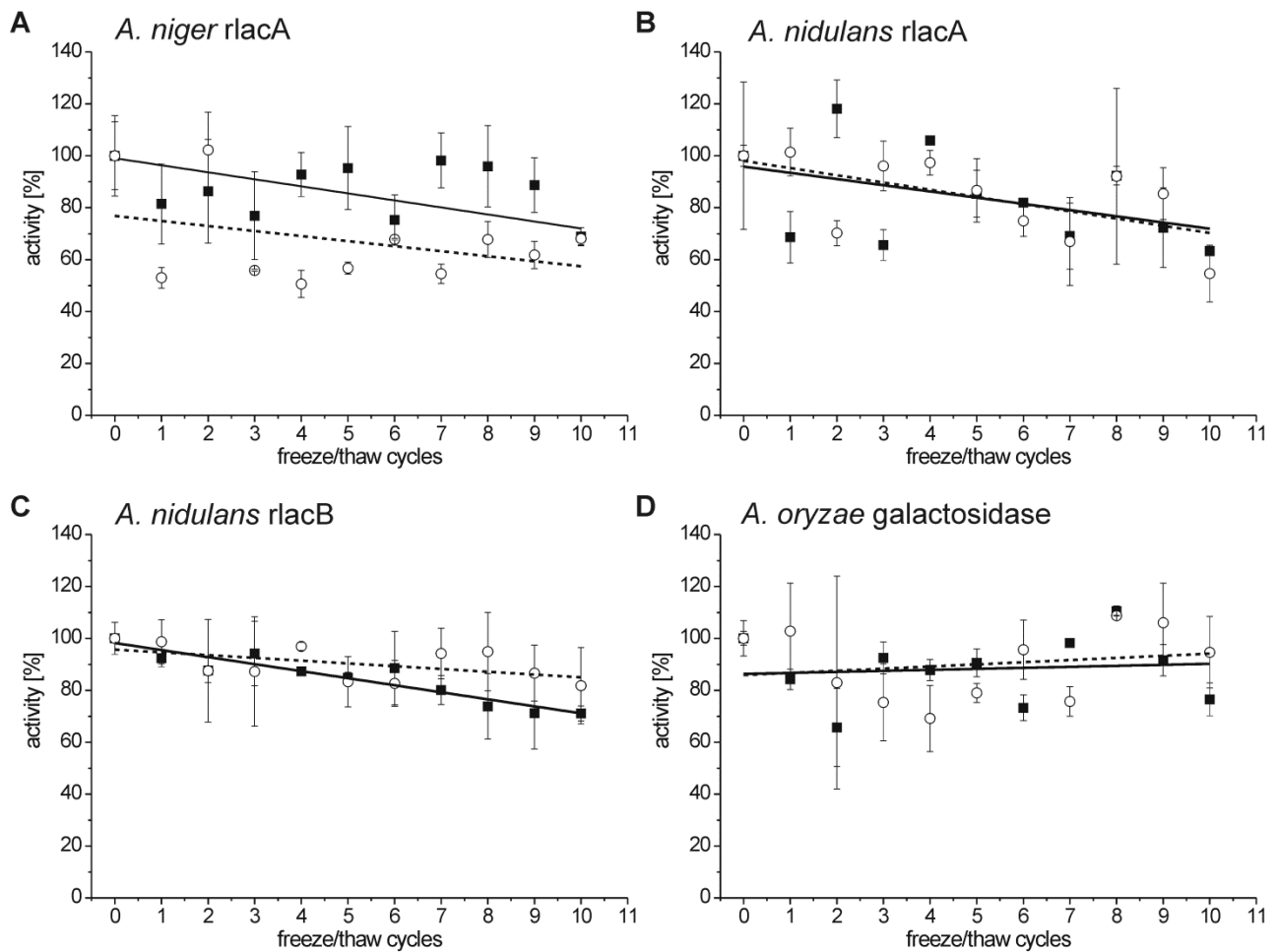
Supplemental Fig. S7 Recombinant *Aspergillus* galactosidases process lactose.

4 μ g of either heat inactivated or functional *A. niger* rlcA, *A. nidulans* rlcA and *A. nidulans* rlcB were used per reaction in a total volume of 50 μ L containing 20% w/v lactose. Incubations were analyzed after 4 h (A) and 24 h (B). The high efficiency of *A. niger* rlcA leads to production of GOS in the first phase of incubation. GOS and lactose are mostly digested into glucose and galactose after 24 hours. The low conversion of lactose when using *A. nidulans* rlcB stems from the low efficiency of this enzyme. Retention times of lactose, galactose, glucose and galacto-oligosaccharides (GOS) are indicated in (A).



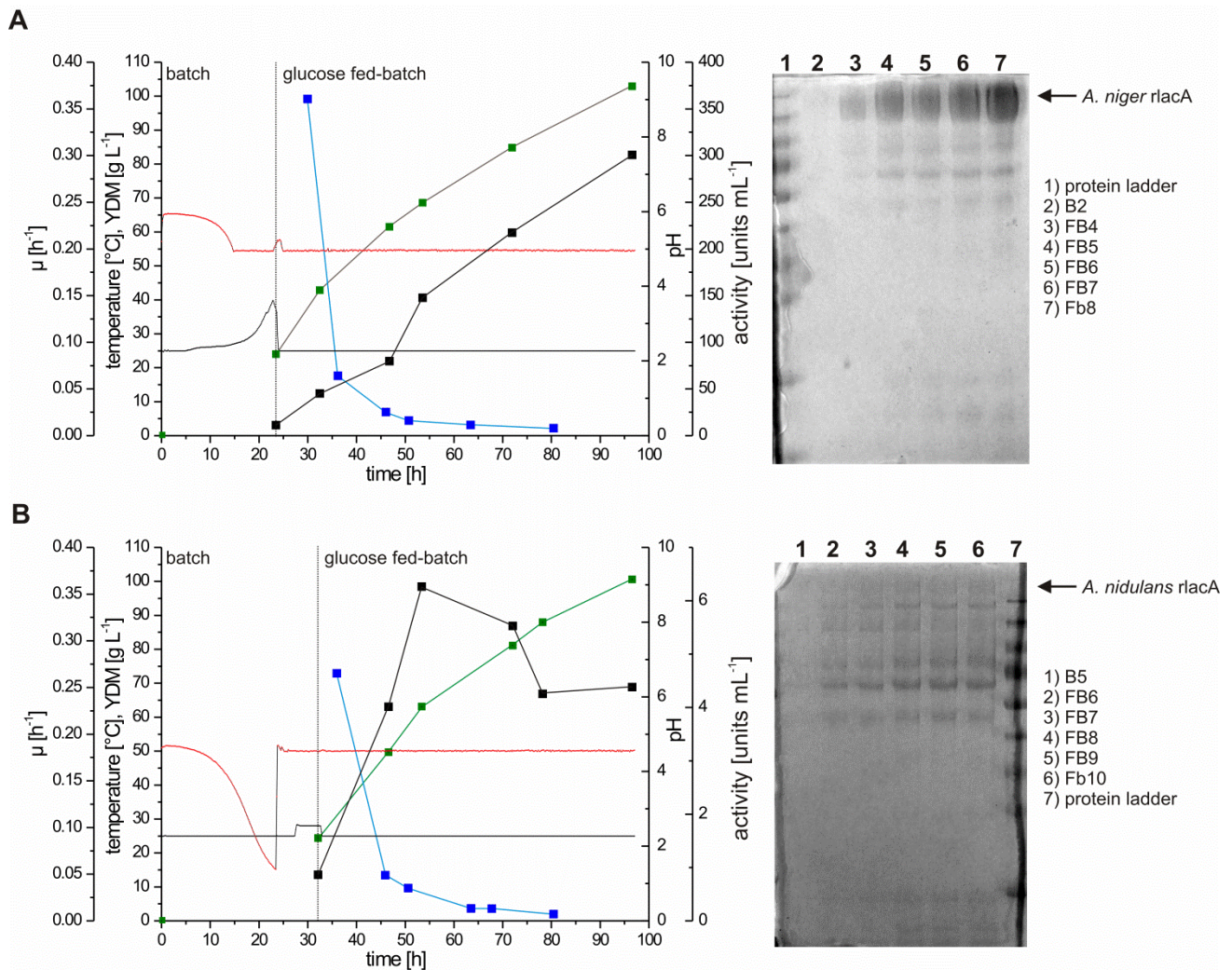
Supplemental Fig. S8 Influence of organic solvents on activity of analyzed β -galactosidases.

Reactions were performed with PNPG as substrate at 37°C with different concentrations of various solvents: (A) acetonitrile, (B) acetone, (C) isopropanol, (D) ethanol, (E) methanol and (F) water at different concentrations. Values represent averages +/- standard error.



Supplemental Fig. S9 Stability of analyzed β -galactosidases subjected to freeze/thaw cycles.

β -galactosidase activity after several freeze/thaw cycles of the respective enzymes at a concentration of $100 \mu\text{g mL}^{-1}$. Data without BSA (black squares, solid trendline) and with BSA (1 mg mL^{-1}) supplementation (open circles, dashed trendline) are shown. Values represent averages \pm standard error.



Supplemental Fig. S10 Summary of bioreactor cultivation of *P. pastoris* X-33 expressing *A. niger* and *A. nidulans* rlacA.

Cultivations were performed essentially as described previously (Gasser, *et al.*, 2010). Figures show the time-course for the production of *A. niger* rlacA (A) and *A. nidulans* rlacA (B). Red lines indicate pH, whereas black lines indicate the temperature throughout the cultivation. Yeast dry mass (YDM) and growth rate (μ) are indicated by green and blue squares, respectively. Black squares indicate β -galactosidase activity in culture supernatant. Total protein samples from culture supernatants were analyzed by SDS-PAGE (on the right in A and B) at the time points indicated in the diagrams. B - batch samples, FB - fed-batch samples. Gels were stained with Coomassie Brilliant Blue.