

Comparative analysis of two members of the metal ion-containing group III-alcohol dehydrogenases from *Dickeya zeae*

Skander Elleuche · Barbara Klippel ·
Amélie von der Heyde · Garabed Antranikian

Received: 7 November 2012 / Accepted: 2 January 2013 / Published online: 30 January 2013
© Springer Science+Business Media Dordrecht 2013

Abstract

Purpose of work A pair of NAD⁺- and NADP⁺-dependent group III-alcohol dehydrogenases was characterized from the enterobacterium, *Dickeya zeae*, to expand our understanding of the distribution and biochemical properties of this interesting group of enzymes.

Two putative group III-alcohol dehydrogenases (ADHs) were identified in the genome of *Dickeya zeae*. Amino acid alignments and phylogenetic analysis revealed that Adh3.1 and Adh3.2 are only distantly related (~25 % identity at the protein level). Both proteins were purified to homogeneity after heterologous expression in *E. coli*. A specific activity of 1.8 U/mg was measured for the NAD⁺-dependent

enzyme Adh3.1 with ethanol used as substrate, while NADPH-dependent Adh3.2 preferred butanal (29.1 U/mg) as substrate. Maximum activity for Adh3.1 was at 50 °C and pH 10 and for Adh3.2 at 70 °C and pH 6. Cell viability assays were used to confirm activity towards butanal and glyoxals. Biochemical characterization and phylogenetic analyses led to the hypothesis that Adh3.1 and Adh3.2 are probably the result of an ancient gene duplication event followed by functional diversification.

Keywords Butanal · *Dickeya zeae* · Ethanol · Gene duplication · Group III-alcohol dehydrogenase · Substrate specificity

Electronic supplementary material The online version of this article (doi:10.1007/s10529-013-1137-2) contains supplementary material, which is available to authorized users.

S. Elleuche · B. Klippel · A. von der Heyde ·
G. Antranikian (✉)
Institute of Technical Microbiology, Hamburg University
of Technology (TUHH), Kasernenstr. 12, 21073
Hamburg, Germany
e-mail: antranikian@tuhh.de

S. Elleuche
e-mail: skander.elleuche@tuhh.de

B. Klippel
e-mail: barbara.klippel@tuhh.de

A. von der Heyde
e-mail: amelievonderheyde@tuhh.de

Introduction

Alcohol dehydrogenases (ADHs) are well-known examples for functional convergent evolution. These enzymes are structurally distinct and show the ability to catalyze similar enzymatic reactions, namely the interconversion of alcohols, aldehydes and ketones. Due to their substrate promiscuity, ADHs are attractive for versatile industrial applications. Beside several NAD(P)⁺-independent ADHs, three families of non-homologous NAD(P)⁺-dependent ADHs are currently accepted (Hernandez-Tobias et al. 2011). Insect-type ADHs contain short peptide chains and do not incorporate a metal ion in their catalytic region. Horse liver ADH (HLADH) is the best-studied

long-chain zinc-dependent ADH (Hernandez-Tobias et al. 2011; Quaglia et al. 2012). Based on amino acid sequence comparisons of metal-ion containing ADHs from thermophilic and mesophilic microorganisms, it was shown that group III-ADHs have risen evolutionarily independently from a common ancestor and are not related to group I- or group II-ADHs, respectively (Radianingtyas and Wright 2003).

As a result of functional diversification, group III metal-containing ADHs can be further divided into several subgroups with distinct substrate specificities, including glycerol dehydratases, propanediol oxidoreductases, lactaldehyde dehydrogenases and others. Due to their ability to process the valuable 1,3-propanediol (1,3-PD), selected members of two subgroups were investigated in detail. The physiological role of DhaT in *Klebsiella pneumoniae* is the recycling of NAD⁺, which is used as co-factor by glycerol dehydrogenase in the oxidative route to produce dihydroxyacetone phosphate, while *yqhD* from *Escherichia coli* has been shown to encode a distantly related unspecific NADPH-dependent aldehyde reductase that is important for the detoxification of harmful aldehydes (Perez et al. 2008; Marcal et al. 2009; Lee et al. 2010; Ma et al. 2010). Nevertheless, both enzymes share the ability to convert 3-hydroxypropionaldehyde into 1,3-PD and are therefore of tremendous interest for applications in biotechnology.

Two distantly related group III-ADHs from the phytopathogenic enterobacterium *Dickeya zeae* were compared in this study. Adh3.1 is a NAD-dependent enzyme preferring short-chain alcohols, while Adh3.2 is a NADPH-dependent aldehyde reductase with activity on butanal and glyoxals. Evolutionary aspects on the distribution in bacteria as well as biochemical properties are reported.

Materials and methods

Strains and culture conditions

Dickeya zeae DSM18068 was obtained from DSMZ (Braunschweig, Germany) and was grown in DSMZ Medium 535. *E. coli* M15[pREP4] in combination with plasmid pQE-30 (Qiagen) was used as heterologous expression host. *E. coli* was grown at 37 °C either in Luria-Bertani (LB) medium or in liquid

fed-batch fermentation medium according to Horn et al. (1996).

Gene cloning procedures

Genomic DNA isolated from *D. zeae* DSM18068 was used as template to amplify ADH-encoding genes *adh3.1* and *adh3.2* by PCR. Primer pairs were as follows: DzDhaT-BamHI-f/DzDhaT-PstI-r (5'-GGA TCCAGCAGTGCATTTTACATTCCCGCC and 5'-CTGCAGTTAGAACGCGGCGGCAAAAATTCCG, restriction sites are underlined) and DzYqhD-BamHI-f/DzYqhD-PstI-r (5'-GGATCCCAGAACTTTACGCT TCATACCCCG and 5'-CTGCAGTTAGCGGGCGGC TTCGTACACGCG). The following sequence was used: 98 °C for 2 min and 35 cycles of 98 °C for 10 s, 60 °C for 20 s and 72 °C for 30 s, and final elongation at 72 °C for 7 min. After subcloning and sequencing, *Bam*HI/*Pst*I digestion fragments were ligated into linearized vector pQE-30 resulting in plasmids pQE30:adh3.1 and pQE30:adh3.2.

Gene expression and purification of proteins

E. coli M15[pREP4] containing plasmid pQE30:adh3.1 or pQE30:adh3.2 was grown in liquid medium (Horn et al. 1996) in a 1.2 l fed-batch fermentation. Expression of ADH-encoding genes was induced with 2 mM IPTG and cells were harvested by centrifugation after 2 h growth. Cells (5 g wet wt) were resuspended in lysis buffer (50 mM NaH₂PO₄ buffer containing 300 mM NaCl and 10 mM imidazole, pH 8.0) and disrupted by Spin Aminco French press (Spectronic Instruments). Purification was achieved in a two-step approach with the ÄKTApurifier system (GE Healthcare): (1) Ni-NTA affinity chromatography with imidazole as elution reagent, followed by (2) size exclusion gel filtration using 50 mM NaH₂PO₄ buffer containing 150 mM NaCl, pH 7.2. Calibration of the size exclusion gel filtration column to determine molecular weights of ADHs was achieved with the following standard proteins according to the information supplied by the manufacturer: ferritin: 440 kDa, aldolase: 158 kDa, conalbumin: 75 kDa and ovalbumin: 44 kDa. Active fractions were pooled, dialyzed against 20 mM Tris buffer (pH 7.0) and stored at 4 °C. SDS-PAGE and Western blotting analysis (His-Tag Monoclonal Antibody, Novagen) were used to visualize the purity of the recombinant ADHs.

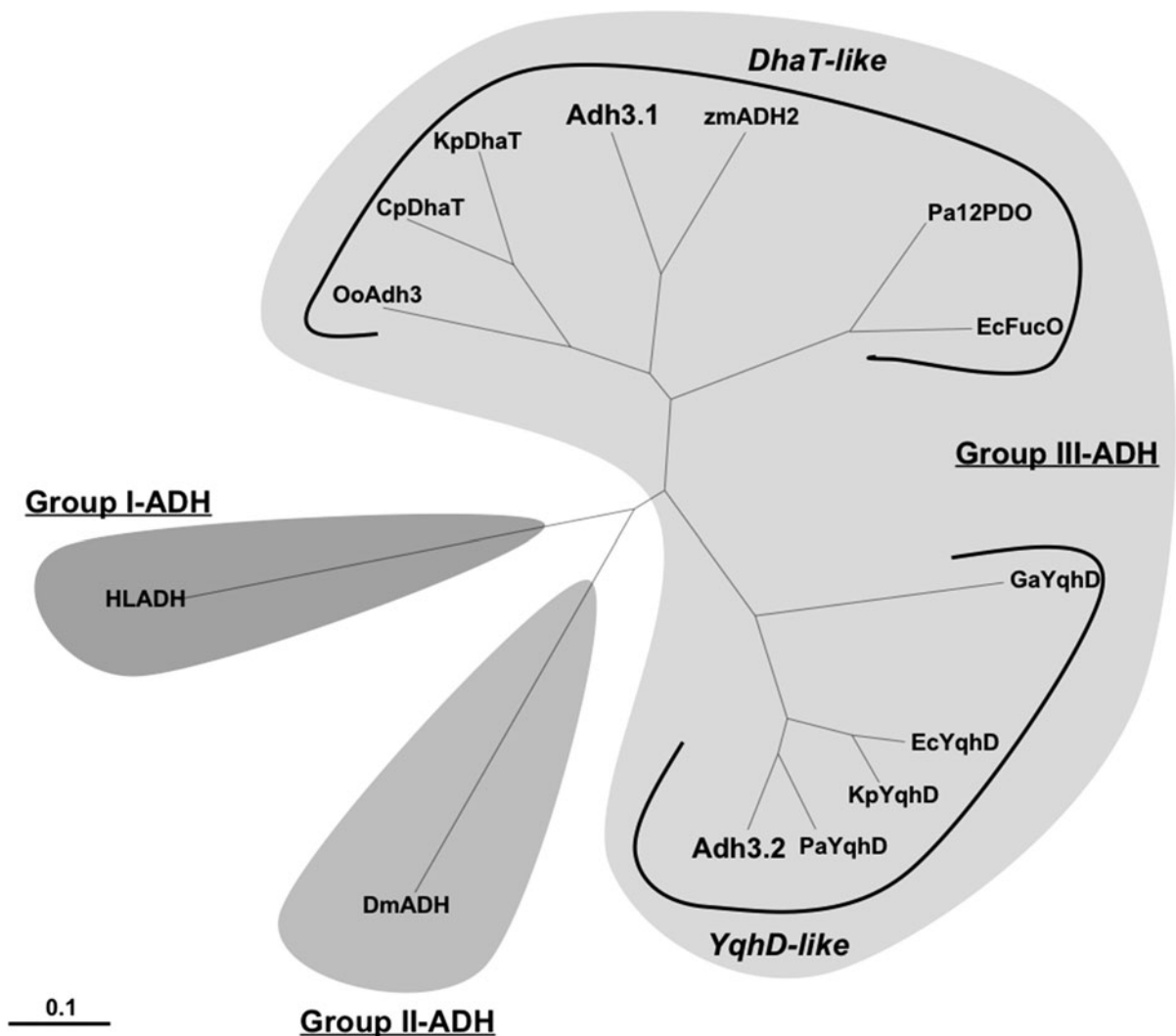


Fig. 1 Unrooted phylogenetic tree separating YqhD and DhaT homologues. The phylogenetic relationship is based on an alignment of complete genes from group I-, II- and III-ADHs (Figure S1). Abbreviations and accession numbers are as follows: *Clostridium pasteurianum* (*CpDhaT*: AF006034), *Dickeya zeae* (*Adh3.1*: HF546061 and *Adh3.2*: HF546062), *Drosophila melanogaster* (*DmADH*: CAA77330.1), *Escherichia coli* (*EcFucO*: AAA23825.1 and *EcYqhD*: NP_417484.1),

Granulicatella adiacens (*GaYqhD*: ZP_05737046.1), *Klebsiella pneumoniae* (*KpDhaT*: YP_005956552.1 and *KpYqhD*: ABR78827.1), *Oenococcus oeni* (*OoAdh3*: HE974350), *Pectobacterium atrosepticum* (*Pa12PDO*: YP_048854.1 and *PaYqhD*: HF546063), *Zymomonas mobilis* (*zmADH2*: BAF76066.1), and horse liver alcohol dehydrogenase (*HLADH*: AAB26666). Group I-ADH HLADH and group II-ADH from *D. melanogaster* were used to illustrate the three groups of ADH

Enzyme assays

Enzyme activities were determined by measuring the reduction of NAD(P)^+ or the oxidation of NAD(P)H at 340 nm in a spectrophotometer equipped with a Peltier effect-controlled temperature cuvette holder. All experiments were performed in triplicate. Standard assays were performed under optimal pH and temperature conditions (*Adh3.1*: pH 10.0; 50 °C and *Adh3.2*:

pH 6.0; 70 °C) and substrates were tested at 10 mM, cofactor concentrations were 0.8 mM NAD^+/NADH for *Adh3.1* and 1 mM NADP/NADPH for *Adh3.2*. One unit of ADH activity is defined as the amount of enzyme needed to catalyze the reduction or formation of 1 μmol NAD(P)^+ per min under standard conditions. In addition, aldehyde reductase activities were indirectly measured in a cell viability assay developed by Lee and Park (2012).

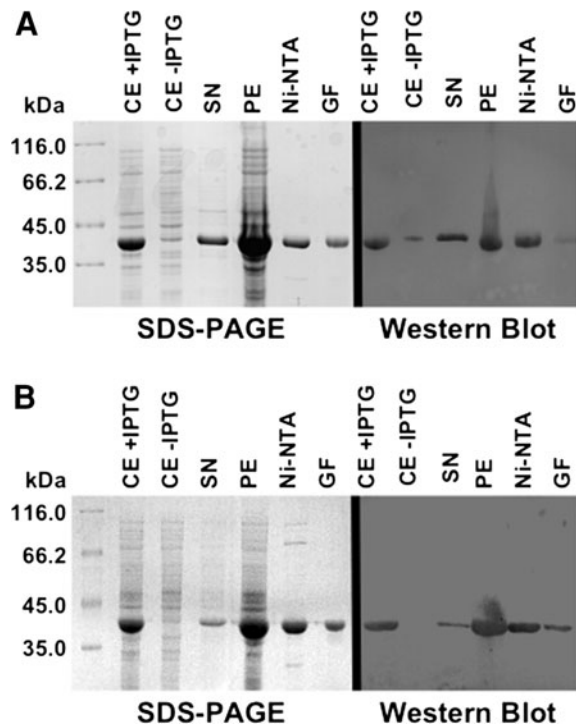


Fig. 2 Expression of *D. zeae adh3.1* and *adh3.2* genes in *E. coli*. SDS-PAGE and western blotting analysis (anti-His antibody) of *E. coli* protein extracts harbouring plasmid pQE30:*adh3.1* (a) or pQE30:*adh3.2* (b), respectively. Cells were induced with 2 mM IPTG (+IPTG) or not induced (–IPTG) and were grown for 2 h at 37 °C. Total cellular proteins (TCP) were resolved by SDS-PAGE and visualized by Coomassie Blue staining. Cells were disrupted and the insoluble cell debris (*Pe* Pellet fraction) was separated by centrifugation from the soluble proteins (*SN* supernatant). His-tagged proteins were purified by affinity chromatography (Ni-NTA) and size exclusion chromatography (SEC)

Sequence accession number

The *adh3.1* and *adh3.2* nucleotide sequences of *D. zeae* strain DSM18068 were deposited under the accession numbers HF546061 and HF546062 in the EMBL database.

Table 2 Substrate specificities of *D. zeae* Adh3.1 and Adh3.2

Substrate ^a	Relative activity [%]	
	Adh3.1-NAD(H)	Adh3.2-NADP(H)
Ethanol ^a	100 ^b	n.d. ^c
Propanol	55 ± 2.9	n.d.
Butanol	n.d.	n.d.
Methanol	n.d.	n.d.
β-Mercaptoethanol	19 ± 0.7	n.d.
Glycerol	n.d.	n.d.
1,3-propanediol	n.d.	n.d.
1,2-propanediol	n.d.	n.d.
Acetaldehyde	46 ± 3.7	n.d.
Propanal	5.3 ± 2.7	n.d.
Butanal	n.d.	100 ^d
Glyoxal	n.d.	15.3 ± 0.2
Methylglyoxal	8.1 ± 2.0	50.4 ± 3.0
Glycoaldehyde	134 ± 0.5	15.4 ± 0.6

^a 10 mM was used as substrate concentration

^b Activity on ethanol (1.8 U/mg) is defined as 100 % in case of Adh3.1

^c n.d. not detectable

^d Activity on butanal (29.1 U/mg) is defined as 100 % in case of Adh3.2

Table 1 Summary of purification steps of recombinant Adh3.1 and Adh3.2 after gene expression in *E. coli* M15[pREP4]

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification factor (fold)
Adh3.1 (crude extract) ^a	133.6	143 ^d	1	100	1
Adh3.1 (Ni-NTA) ^b	109.8	125	1.2	87	1.2
Adh3.1 (SEC) ^c	26.5	51.3	1.8	36	1.8
Adh3.2 (crude extract) ^a	127.2	580 ^e	4.6	100	1
Adh3.2 (Ni-NTA) ^b	12.7	283	22.2	49	4.8
Adh3.2 (SEC) ^c	8.5	248	29.1	43	6.3

^a Cells were disrupted by French Press, cell debris was sedimented

^b Purified protein fraction using Ni-NTA column at room temperature

^c Purified protein fraction using size exclusion chromatography

^d Activity of Adh3.1 was measured at pH 10.0 and 50 °C with 10 mM ethanol and 0.8 mM NAD⁺

^e Activity of Adh3.2 was measured at pH 6.0 and 70 °C with 10 mM butanal and 1 mM NADPH

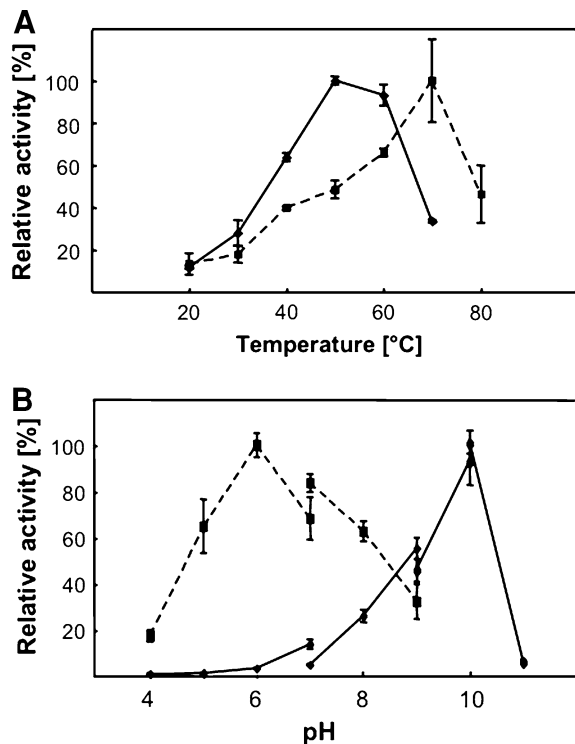


Fig. 3 Effect of temperature and pH on ADH activity. **a** Temperature. Purified Adh3.1 (filled diamonds, black line) was tested at different temperatures under optimal pH conditions with EtOH as substrate. Adh3.2 was tested using butanal (filled squares, dashed line) as substrate at different temperatures. **b** For the determination of the pH-optimium, Adh3.1 was incubated in citrate-phosphate buffer (pH 4 to 7, filled diamonds, black line), Tris buffer (pH 7–9, filled diamonds, black line), Glycine NaOH buffer (pH 9–10, filled diamonds, black line) and CAPS buffer (pH 10–11, filled diamonds, black line) using ethanol as substrate at 50 °C. The enzyme activity with butanal (filled squares) was measured at 70 °C in citrate-phosphate buffer (pH 4–7, dashed line) and Tris buffer (pH 7–9, dashed line)

Results

Sequence analysis and phylogenetic relationships of alcohol dehydrogenases

To investigate multiple members of group III-ADHs from a single microorganism, BlastP-analyses were performed using amino acid sequences of characterized alcohol dehydrogenases, 1,3-propanediol oxidoreductases and aldehyde reductases from various bacterial species (Altschul et al. 1990). Two diverse genes encoding putative group III-ADHs were identified within the sequenced genome of *D. zeae* Ech1591 and were

Table 3 Kinetic constants of Adh3.1 and Adh3.2 from *D. zeae*

Adh3.1	
Ethanol - K_M (mM)	14
Ethanol - v_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	3.4
Acetaldehyde - K_M (mM)	7.9
Acetaldehyde - v_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	7.5
NAD^+ - K_M (mM)	0.5
NAD^+ - v_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	3
NADH - K_M (mM)	0.6
NADH - v_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	4.2
Adh3.2	
Butanal - K_M (mM)	1.3
Butanal - v_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	45
NADPH - K_M (mM)	0.4
NADPH - v_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	68

amplified by PCR from genomic DNA of *D. zeae* DSM18068. The 1,158 bp ORF *adh3.1* encodes a protein of 385 amino acids, while the protein Adh3.2 is composed of 387 amino acids and encoded by *adh3.2* (1,164 bp). The amino acid identity between both proteins is rather low with 24.9 % in a 349 amino acid overlap. InterProScan assigned both proteins to the group of iron-type ADHs (Quevillon et al. 2005). Phylogenetic analysis also separated both proteins from each other (Fig. 1). Moreover, the unrooted tree clearly opposed group III-ADHs to prototypes of group I or group II, respectively. Adh3.1 belongs to group III-ADHs with sequence identity to proteins involved in lactaldehyde metabolism and production of 1,2-propanediol and 1,3-propanediol. DhaT (49.3 % identity to Adh3.1) from *K. pneumoniae* and FucO (42.4 % identity to Adh3.1) from *E. coli* are well-investigated members of this subgroup. However, Adh3.2 belongs to the subgroup of putative butanol dehydrogenases within the group III-ADHs with *E. coli* YqhD being the characterized prototype. Adh3.2 shares 77.3 % identity with YqhD.

Expression and purification of recombinant Adh3.1 and Adh3.2

Both putative group III-ADHs from *D. zeae* were cloned into pQE30 expression vector and the genes were expressed in *E. coli* M15[pREP4]. Proteins were purified in a 2-step purification approach and visualized on SDS-PAGE and by western blotting analyses (Fig. 2). Expression of *adh3.1* and *adh3.2* gave a single

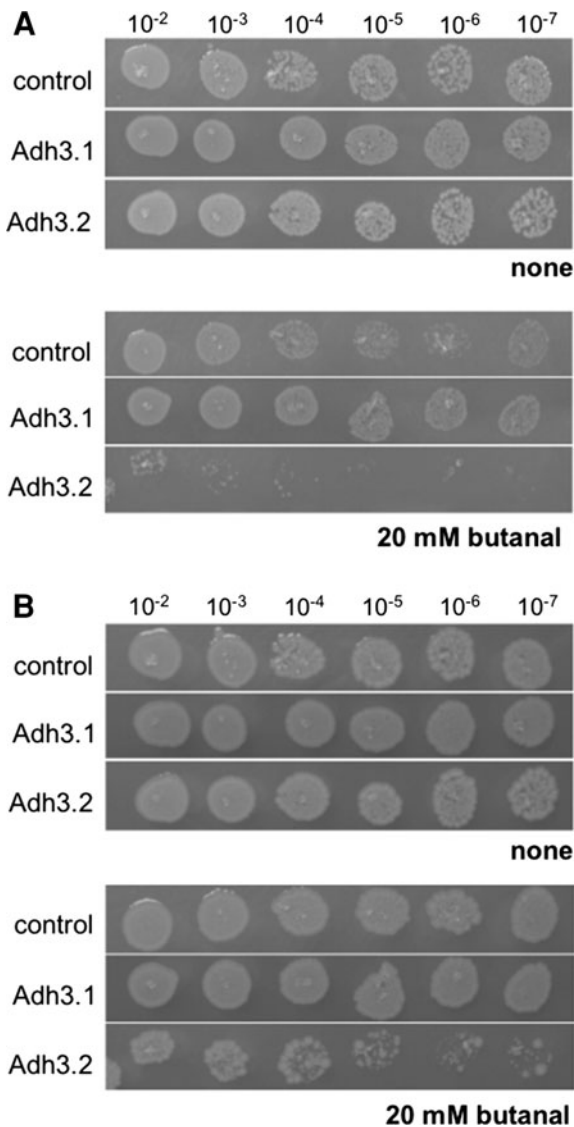


Fig. 4 Cell viability assay to measure susceptibility to butanal of *E. coli* expressing *adh3.1* or *adh3.2*, respectively. **a** Pictures were taken after 24 h of growth. **b** Pictures were taken after 48 h of growth. Spot assays were performed with *E. coli* M15[pREP4] either harbouring mock vector pQE-30 (*control*) or pQE30:*adh3.1* or pQE30:*adh3.2*

band of $\sim 44\text{--}41$ kDa, which is in good agreement with the joined molecular masses of proteins (Adh3.1: 43.4 kDa and Adh3.2: 41.3 kDa) and RGS-6xHis-tags (1.1 kDa). Enzymatic activities of 1.8 U/mg (Adh3.1) and 29.1 U/mg (Adh3.2) were obtained from purified enzymes after size exclusion chromatography with ethanol or butanal, respectively (Table 1). The calculated molecular weights from native size exclusion chromatography are 70.5 ± 2.8 kDa (Adh3.1) and

68.4 ± 0.7 kDa (Adh3.2), indicating that both proteins form dimeric structures (Fig. S2).

Enzymatic properties of alcohol dehydrogenases

Enzyme specificities are given in Table 2. Activity measurements revealed that Adh3.1 is active on a broad range of short-chain alcohols with NAD^+ being an appropriate co-factor, while Adh3.2 is an NADPH-dependent aldehyde reductase with specific activity towards butanal and 2-oxoaldehydes. Adh3.1 showed optimal activity at 50 °C and pH 10.0, while Adh3.2 is optimally active at 70 °C and pH 6.0 (Fig. 3). To study the kinetic constants of the enzymes with their preferred substrates, non-linear regression analysis of the corresponding Michaelis-Menten curves was performed (Table 3). Sensitivity to butanal and resistance to glyoxal and methylglyoxal of *E. coli* producing Adh3.1 and Adh3.2 was further tested in plate assays. Expression of *adh3.2* completely blocked growth of *E. coli* on plates containing 20 mM butanal, indicating the production of toxic butanol, whereas expression of *adh3.1* allowed the cells to survive (Fig. 4). *E. coli* expressing either *adh3.1* or *adh3.2*, respectively, exhibited a higher resistance to methylglyoxal (Fig. 5a), and the production of Adh3.2 led to a decomposition of toxic glyoxal (Fig. 5b), which is in good agreement with the enzyme activity towards these compounds (Table 2).

Discussion

ADH is an excellent example of a versatile enzyme family that has developed diverse functionalities. These proteins execute multiple physiological roles in all organisms by catalyzing the interconversion of alcoholic compounds and aldehydes or ketones. So far, three evolutionary independent groups of NAD(P)-dependent ADHs were described in pro- and eukaryotes and each group contains several subgroups of specialized enzymes probably evolved from ancestral tandem gene duplication and neofunctionalization events. Gene duplication is crucial to functional innovation and has been shown to appear in an unexpectedly high rate in transcription factors, transporters and enzymes. Gene duplication followed by functional innovation has been described for a group-II ADH of *Drosophila melanogaster*, in which the first of the duplicated genes

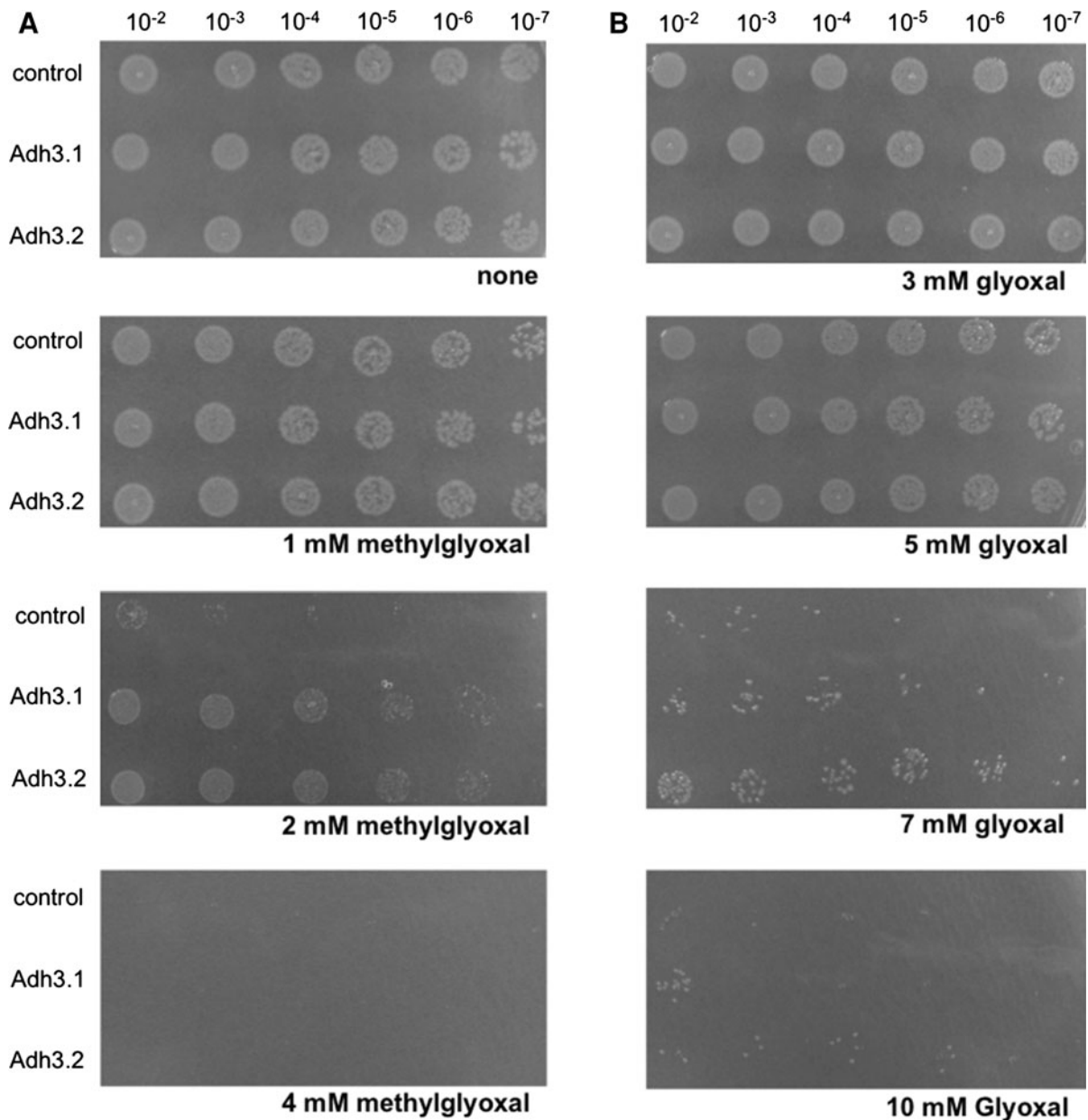


Fig. 5 Cell viability assay to measure resistance to glyoxals. **a** LB medium supplemented with different concentrations of methylglyoxal. Pictures were taken after 24 h of growth. **b** Growth assays were performed on LB medium supplemented

with glyoxal. Pictures were taken after 24 h of growth. Spot assays were performed with *E. coli* M15[pREP4] either harbouring mock vector pQE-30 (*control*) or pQE30:adh3.1 or pQE30:adh3.2

codes for an enzyme optimized for the reduction of acetaldehyde and the second for the reverse reaction (Conant and Wolfe 2008). Although several members of group III-ADHs have been investigated, little information is available about evolutionary aspects and distribution of genes encoding group III-ADHs. Despite

being characterized from *E. coli* and *K. pneumoniae* in detail, DhaT- and YqhD-like proteins were mainly used to establish recombinant strains for biotechnological applications including the production of valuable chemical compounds (Xiu and Zeng 2008; Jarboe 2011).

Biochemical and phylogenetic characterization of two distantly related group III-ADHs from the plant-pathogenic enterobacterium *D. zea* revealed that these enzymes have undergone an interesting evolution resulting in different substrate specificities and cofactor-dependency. Adh3.1 was a NAD-dependent ADH with preference for ethanol, while Adh3.2 preferred the phosphorylated cofactor and was mostly active on butanal. The ability of Adh3.1 and Adh3.2 to reduce aldehyde compounds was also confirmed by cell viability assays on plates containing butanal, glyoxal or methylglyoxal, respectively. Recently two ADHs from the extreme halophile, *Haloferax volcanii*, have been compared with regards to their biochemical properties after homologous overexpression in the native host and were shown to accept highly diverse substrates and displayed different stability and activity profiles (Timpson et al. 2012). These isozymes share an identity of 31.5 % at the protein level and belong to group I of ADHs.

The formation of dimers, which has been observed by size exclusion chromatography, is well-known for group III-ADHs and has been shown for several isozymes including *E. coli* FucO, *E. coli* YqhD and *Zymomonas mobilis* zmADH2, respectively (Sulzenbacher et al. 2004; Montella et al. 2005; Moon et al. 2011). So far, structure determination indicated that DhaT-like enzymes contain Fe²⁺ in their catalytic region, while *E. coli* YqhD is Zn²⁺-dependent. Since Adh3.1 and Adh3.2 exhibit all conserved key residues in their primary structures, it can be speculated that these proteins are metallo-enzymes as well (Supplementary Figure 1). Another indication of the presence of a metal-ion in the protein structures is the inactivation during Ni-NTA purification (data not shown). Since imidazole is a chelating agent, elution of metallo-enzymes from Ni-NTA columns might interfere with catalytic activity (Quaglia et al. 2012) However, dialyzing the protein sample against imidazole-free buffer prior to size exclusion chromatography reversed inhibition in case of Adh3.1.

This study expands our understanding of the distribution and biochemical properties of group III-ADHs in enterobacteria. A novel NAD-dependent ADH and a NADPH-dependent aldehyde reductase were characterized from *D. zea*. Both enzymes were expressed at a high level in a heterologous host in *E. coli* and were purified with a simple 2-step purification procedure. Moreover, one enzyme was

active on a range of alcohol and aldehyde substrates, while the second enzyme was only active towards aldehydes.

Acknowledgments The authors thank Henning Piascheck for the help with the fermentation experiments. This work was funded by the Excellence Cluster in the Excellence Initiative by the State of Hamburg “Fundamentals of Synthetic Biological Systems (SynBio)”.

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Conant GC, Wolfe KH (2008) Turning a hobby into a job: how duplicated genes find new functions. *Nat Rev Genet* 9:938–950
- Hernandez-Tobias A, Julian-Sanchez A, Pina E, Riveros-Rosas H (2011) Natural alcohol exposure: is ethanol the main substrate for alcohol dehydrogenases in animals? *Chem Biol Interact* 191:14–25
- Horn U, Strittmatter W, Krebber A, Knupfer U, Kujau M, Wenderoth R, Muller K, Matzku S, Pluckthun A, Riessenberg D (1996) High volumetric yields of functional dimeric miniantibodies in *Escherichia coli*, using an optimized expression vector and high-cell-density fermentation under non-limited growth conditions. *Appl Microbiol Biotechnol* 46:524–532
- Jarboe LR (2011) YqhD: a broad-substrate range aldehyde reductase with various applications in production of bio-renewable fuels and chemicals. *Appl Microbiol Biotechnol* 89:249–257
- Lee C, Park C (2012) Development of a suicidal vector-cloning system based on butanal susceptibility due to an expression of YqhD aldehyde reductase. *J Microbiol* 50:249–255
- Lee C, Kim I, Lee J, Lee KL, Min B, Park C (2010) Transcriptional activation of the aldehyde reductase YqhD by YqhC and its implication in glyoxal metabolism of *Escherichia coli* K-12. *J Bacteriol* 192:4205–4214
- Ma C, Zhang L, Dai J, Xiu Z (2010) Relaxing the coenzyme specificity of 1,3-propanediol oxidoreductase from *Klebsiella pneumoniae* by rational design. *J Biotechnol* 146:173–178
- Marcal D, Rego AT, Carrondo MA, Enguita FJ (2009) 1,3-Propanediol dehydrogenase from *Klebsiella pneumoniae*: decameric quaternary structure and possible subunit cooperativity. *J Bacteriol* 191:1143–1151
- Montella C, Bellsollell L, Perez-Luque R, Badia J, Baldoma L, Coll M, Aguilar J (2005) Crystal structure of an iron-dependent group III dehydrogenase that interconverts L-lactaldehyde and L-1,2-propanediol in *Escherichia coli*. *J Bacteriol* 187:4957–4966
- Moon JH, Lee HJ, Park SY, Song JM, Park MY, Park HM, Sun J, Park JH, Kim BY, Kim JS (2011) Structures of iron-dependent alcohol dehydrogenase 2 from *Zymomonas mobilis* ZM4 with and without NAD⁺ cofactor. *J Mol Biol* 407:413–424
- Perez JM, Arenas FA, Pradenas GA, Sandoval JM, Vasquez CC (2008) *Escherichia coli* YqhD exhibits aldehyde reductase

- activity and protects from the harmful effect of lipid peroxidation-derived aldehydes. *J Biol Chem* 283:7346–7353
- Quaglia D, Irwin JA, Paradisi F (2012) Horse liver alcohol dehydrogenase: new perspectives for an old enzyme. *Mol Biotechnol* 52:244–250
- Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R (2005) InterProScan: protein domains identifier. *Nucleic Acids Res* 33:W116–W120
- Radianingtyas H, Wright PC (2003) Alcohol dehydrogenases from thermophilic and hyperthermophilic archaea and bacteria. *FEMS Microbiol Rev* 27:593–616
- Sulzenbacher G, Alvarez K, Van Den Heuvel RH, Versluis C, Spinelli S, Campanacci V, Valencia C, Cambillau C, Eklund H, Tegoni M (2004) Crystal structure of *E. coli* alcohol dehydrogenase YqhD: evidence of a covalently modified NADP coenzyme. *J Mol Biol* 342:489–502
- Timpson LM, Liliensiek AK, Alsafadi D, Cassidy J, Sharkey MA, Liddell S, Allers T, Paradisi F (2012) A comparison of two novel alcohol dehydrogenase enzymes (ADH1 and ADH2) from the extreme halophile *Haloferax volcanii*. *Appl Microbiol Biotechnol* 97(1):195–203
- Xiu ZL, Zeng AP (2008) Present state and perspective of downstream processing of biologically produced 1,3-propanediol and 2,3-butanediol. *Appl Microbiol Biotechnol* 78:917–926