Microbiological Research 194 (2017) 47–52

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Short Communication

The aldehyde dehydrogenase, AldA, is essential for L-1,2-propanediol utilization in laboratory-evolved *Escherichia coli*

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ARTICLE INFO

Article history:
Received 21 October 2016
Accepted 29 October 2016
Available online 4 November 2016

Keywords:
Microbial metabolilsm
Systems biology
Genome-scale models
Adaptive laboratory evolution
Pathway gap filling

ABSTRACT

Most *Escherichia coli* strains are naturally unable to grow on 1,2-propanediol (PDO) as a sole carbon source. Recently, however, a K-12 descendent *E. coli* strain was evolved to grow on 1,2-PDO, and it was hypothesized that this evolved ability was dependent on the aldehyde dehydrogenase, AldA, which is highly conserved among members of the family Enterobacteriacea. To test this hypothesis, we first performed computational model simulation, which confirmed the essentiality of the *aldA* gene for 1,2-PDO utilization by the evolved PDO-degrading *E. coli*. Next, we deleted the *aldA* gene from the evolved strain, and this deletion was sufficient to abolish the evolved phenotype. On re-introducing the gene on a plasmid, the evolved phenotype was restored. These findings provide experimental evidence for the computationally predicted role of AldA in 1,2-PDO utilization, and represent a good example of *E. coli* robustness, demonstrated by the bacterial deployment of a generalist enzyme (here AldA) in multiple pathways to survive carbon starvation and to grow on a non-native substrate when no native carbon source is available.

1. Introduction

Advances in sequencing technology and accumulation of genomic information accelerated the discovery and exploitation of novel pathways in medically and industrially important microorganisms. Nevertheless, even 20 years after the first microbial genome was sequenced (Fleischmann et al., 1995), the encoded functions of over 40% of sequenced microbial genes remain undetermined (Aziz et al., 2012). Even more intriguingly, novel functions are still being discovered for genes with previously established roles, as revealed by systematic gene deletion investigations (e.g., Baba et al., 2006) and adaptive laboratory evolution (ALE) experiments (Conrad et al., 2011).

As an example, one experimentally well-studied pathway that is still being explored on the genomic level is the propanediol utilization pathway. 1,2-propanediol (PDO: PubChem ID: 1030, CAS# 57-55-6), also known as propylene glycol, is a water-miscible organic solvent commonly used in industrial and medical applications (O'Neil, 2006). In industry, 1,2-PDO is a substrate in polymer production and an approved food additive (E# 1520) (Niu and Guo, 2015). In medicine, it is used as an antiseptic, a vehicle for intravenous and dermatological formulations, as well as a hygroscopic agent in respiratory inhalants (Bennett and San, 2001). Moreover, there is mounting evidence that 1,2-PDO plays a key role in the metabolic activities of human gut microbiota (Pacheco et al., 2012; Staib and Fuchs, 2014).

Theoretically, *E. coli* has the potential to catabolize 1,2-PDO through one of two pathways. The first involves the propanediol-utilization (PDU) locus, which only some strains possess (Monk et al., 2013). The second has been hypothesized to involve the aldehyde dehydrogenase, *aldA*. Several research groups have suggested a key role for this gene since AldA is the only enzyme in the *E. coli* genome annotated to oxidize the intermediate product L-lactaldehyde to L-lactate (Hacking et al., 1978; Chen et al., 1987; Zhang et al., 2006). No definitive experimental proof, however, has

Abbreviations: 1,2-PDO, 1,2-propanediol; ALE, Adaptive laboratory evolution; K12PDO, Evolved *E. coli* K-12 capable of growth on L-1,2-PDO.

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http://dx.doi.org/10.1016/j.micres.2016.10.006
0944-5013/

been provided to support this hypothesis, in large part because this AldA-associated pathway is not active in wild-type *E. coli* isolates, which naturally fail to utilize 1,2-PDO as a carbon source.

A recent study reported the use of ALE to generate a strain of *E. coli* K-12 MG1655 that can grow in defined media with L-1,2-PDO as the sole carbon source and in the absence of the PDU locus (Lee and Palsson, 2010); yet, the biochemical basis of the evolved mechanism was not fully deciphered. Here, we used this laboratory-evolved strain to test and confirm the hypothesis that *aldA* does indeed play a key role in 1,2-PDO utilization through computational modeling and experimentation

2. Materials and methods

2.1. Bacterial strains and culture conditions

Two *Escherichia coli* K-12 MG1655 strains were used in this study: the wild type and a descendant that had been evolved to utilize 1,2-PDO as the primary carbon source (Lee and Palsson, 2010). Bacteria were maintained on Luria Bertani (LB) broth or plates. Carbon source and growth curve experiments were performed in M9 minimal media (Fong et al., 2013) supplemented with glucose (Sigma, St. Louis, MO) M9-glucose, or with L-1,2-propanediol (Sigma, St. Louis, MO), M9-PDO. In M9-glucose, glucose was added at 2 g/L concentrations, whereas L-1,2-PDO was added to M9-PDO at 1, 2, 4, or 8 g/L according to the experimental design. Ampicillin, kanamycin, and chloramphenicol (Sigma, St. Louis, MO) were added for selection of transformed strains to final concentrations of 100, 50, and 25 ug/mL, respectively.

Cultures were routinely tested for purity on MacConkey or Eosin-Methylene Blue agar plates.

2.2. Plasmids

pKD46, pKD13, pCP20 were used for precise gene deletion by the Lambda Red system according to Baba and coworkers' modification (Baba et al., 2006) of the original protocol described by Datsenko and Warner (Datsenko and Wanner, 2000). pASK1988 was used for gene complementation exactly as previously described (Fong et al., 2013).

2.3. Computational genomic screening and pathway analysis

Genomic screening for AldA homologs and paralogs was performed on the SEED genome analysis servers (Aziz et al., 2012). Specifically, the "Compare Region" tool in the SEED server was used to find major homologs of the *E. coli* AldA-encoding gene; then all confirmed orthologs of the gene (defined as bidirectional best hits) were downloaded from the SEED database. Absence of other *aldA* copies in *E. coli* and absence of the gene was investigated by the "Browse Subsystems" feature in the SEED database (Aziz et al., 2012). Further sequence analysis and confirmation of gene presence/absence were performed by BlastP and tBLastN (Altschul et al., 1997).

The COBRApy toolbox (Ebrahim et al., 2013) and Escher visualization tool (King et al., 2015) were used for pathway investigation of the role of AldA in PDO utilization.

2.4. Construction of deletion mutants and genetic complementation

The Lambda Red system, originally developed by Datsenko and Warren (Datsenko and Wanner, 2000), was used for precise gene deletion according to the modified protocol used in creating the Keio collection (Baba et al., 2006). Primers used for construction of the knockout strain and confirmation of *aldA* deletion have been described in our recent work (Aziz et al., 2015b). Genetic complementation with *aldA*-carrying pASK plasmid was used exactly as described (Fong et al., 2013).

2.5. Analysis of aldA transcription by real-time reverse transcriptase polymerase chain reaction (real-time RT PCR)

RT-PCR experiments and measurements were carried out in a CFX96 C1000 Touch instrument running CFX Manager software version 3.0.1224.1015 (Bio-Rad Hercules, CA). All experiments were in accordance with the MIQE guidelines (Bustin et al., 2009; Bustin et al., 2013).

RNA was extracted by the RNeasy kit (Qiagen, Valencia, CA) from three biological replicates of each strain grown to mid-log phase. RNA concentration and quality were checked by a NanoDrop instrument (Thermo Fisher, Waltham, MA).

SuperScript III reverse transcriptase was used for first strand cDNA synthesis, in presence of 10 mM dNTPs, random primers, DTT, 1 st strand buffer, the RNase inhibitor, Superase-In, and DEPC-treated water, all purchased from Life Technologies (Carlsbad, CA). Thermocycler conditions for annealing of random primers were 70 °C for 10 min; 25 °C for 10 min; 4 °C hold. The mixture was subsequently added in its entirety to reagents for first strand synthesis, for which the thermocycler conditions were: 25 °C for 10 min; 37 °C for 60 min; 42 °C for 60 min; 70 °C for 10 min; 4 °C hold. Next, 20 μL of a 1 N NaOH solution was added to the mixture and incubated at 65 °C for 30 min, after which 20 μL of a 1 N HCl solution was added to neutralize. The cDNA was then purified with the Qiagen PCR purification kit. The quantity and purity were again measured in Nanodrop and Qubit instruments (Thermo Fisher, Waltham, MA), and the integrity was measured with a Bioanalyzer (Agilent Technologies, Santa Clara, CA).

The housekeeping genes used for normalization were *ihfB*, *hcaT*, and *idnT* (Zhou et al., 2011). Primer sequences and product lengths for these three genes, plus that for *aldA*, are provided in Table 1.

The RT-PCR cycling conditions were: 98 °C for 6 min followed by 65 cycles of 98 °C for 15 s, 55 °C for 15 s. The reagents were 1 μL each of the forward and reverse primers in which the stock concentrations were 1 μM, 5 μL of SsoAdvanced SYBR green mix (Bio-Rad, Hercules, CA), 10 ng of cDNA, and water up to 10 μL final volume.

A similar mixture was used to determine the efficiency for each primer set, but genomic DNA (gDNA) replaced the cDNA and a series of five serial dilutions were used. The highest concentration of gDNA was 10 ng, and each of the dilutions differed in concentration by 1/10. An additional well containing no gDNA was also added to the melt curve analysis.

The threshold cycle (C_t) for each sample was calculated using the single threshold, baseline subtracted curve fit algorithm supplied with the CFX Manager software. C_t values for no template control (NTC) wells were routinely 35 and higher, whereas for sample wells, these values ranged between 10 and 20. Normalization of the *aldA* transcript levels to the housekeeping genes was also calculated with the same software.

2.6. Phenotypic screens and growth experiments

Phenotypic screens were performed on LB, M9, M9-glucose, or M9-PDO. All growth experiments were performed in replicates (3–6 technical replicates and at least two biological replicates) in 100-well plates that were loaded into the Bioscreen C instrument (Oy Growth Curves Ab Ltd, Finland) and measured automatically every 20 min at 37° C for up to 48 h, after 10-s shaking prior to each measurement. Readings were copied to Microsoft Excel, and the growth rate was calculated as the slope of the logarithmic best-fit line during exponential growth.