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BIOTECHNOLOGICALLY RELEVANT ENZYMES AND PROTEINS

Improving the acetic acid tolerance and fermentation of *Acetobacter pasteurianus* by nucleotide excision repair protein UvrA

Yu Zheng[1] · Jing Wang[1] · Xiaolei Bai[1] · Yangang Chang[1] · Jun Mou[1] · Jia Song[1] · Min Wang[1]

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Abstract

Acetic acid bacteria (AAB) are widely used in acetic acid fermentation due to their remarkable ability to oxidize ethanol and high tolerance against acetic acid. In *Acetobacter pasteurianus*, nucleotide excision repair protein UvrA was up-regulated 2.1 times by acetic acid when compared with that without acetic acid. To study the effects of UvrA on *A. pasteurianus* acetic acid tolerance, *uvrA* knockout strain AC2005-ΔuvrA, uvrA overexpression strain AC2005 (pMV24-*uvrA*), and the control strain AC2005 (pMV24), were constructed. One percent initial acetic acid was almost lethal to AC2005-Δ*uvrA*. However, the biomass of the UvrA overexpression strain was higher than that of the control under acetic acid concentrations. After 6% acetic acid shock for 20 and 40 min, the survival ratios of AC2005 (pMV24-*uvrA*) were 2 and 0.12%, respectively; however, they were 1.5 and 0.06% for the control strain AC2005 (pMV24). UvrA overexpression enhanced the acetification rate by 21.7% when compared with the control. The enzymes involved in ethanol oxidation and acetic acid tolerance were up-regulated during acetic acid fermentation due to the overexpression of UvrA. Therefore, in *A. pasteurianus*, UvrA could be induced by acetic acid and is related with the acetic acid tolerance by protecting the genome against acetic acid to ensure the protein expression and metabolism.

Keywords Acetic acid tolerance · *Acetobacter pasteurianus* · Nucleotide excision repair protein · Acetic acid fermentation · Genome damage

Introduction

Acetic acid is a highly important organic acid that is widely used in chemistry, medicine, and food industry. Acetic acid has an inhibitory effect on some microorganism growth and metabolism when its concentration reaches 5 g/L. Acetic acid bacteria (AAB) are gram-negative, aerobic bacteria belonging to the *Acetobacteraceae* family. The remarkable ability of AAB to oxidize ethanol and high tolerance to acetic acid makes it widely used in the acetic acid fermentation industry (Hattori et al. 2011; Sengun and Karabiyikli 2011). In addition to acetic acid, AAB are important functional microorganisms in the production of different vinegars, including traditional Chinese cereal vinegars that are generally produced through solid-state fermentation, traditional European fruit vinegars produced through liquid-surface fermentation, and the vinegar produced in liquid-submerged fermentation with pure strains of AAB (Solieri and Giudici 2009; Nie et al. 2015).

A high acetic acid tolerance of AAB is crucial for industrial acetic acid and vinegar production. According to previous reports, the acetic acid tolerance mechanism of AAB is mainly related to (i) ethanol oxidation by membrane-bound alcohol dehydrogenase (ADH) (Trcek et al. 2007; Trcek et al. 2006)—the high ADH activity in the *Gluconacetobacter* cells and high acetic acid stability of the enzyme enable these species to grow and stay metabolically active at 10% acetic acid (Trcek et al. 2006); (ii) tricarboxylic acid (TCA) cycle involving citrate synthase (CS) and aconitase (Fukaya et al. 1990); (iii) the acetic acid assimilation by transferase of acetyl coenzyme AarC (Mullins et al. 2008); (iv) the ATP-binding cassette (ABC) transporter playing a role in pumping the acetic acid out of the AAB (Nakano and Fukaya 2008), (v) press tolerance proteins induced by acetic acid, such as molecular chaperones DnaK and GroEL, to ensure proper protein folding in adverse environments—co-overexpression of GrpE

✉ Min Wang
minw@tust.edu.cn

[1] State Key Laboratory of Food Nutrition and Safety; Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education; Tianjin Engineering Research Center of Microbial Metabolism and Fermentation Process Control; College of Biotechnology, Tianjin University of Science & Technology, Tianjin 300457, People's Republic of China

with DnaK/J in *Acetobacter pasteurianus* resulted in an improved growth under different pressure conditions (Ishikawa et al. 2010; Okamoto-Kainuma et al. 2004); (vi) changes in cell morphology and membrane composition and the pellicle (capsular polysaccharides) formation (Trcek et al. 2007); and (vii) the metabolism of some amino acids (Akiko et al. 2002; Ishikawa et al. 2010; Okamoto-Kainuma et al. 2004).

The weak electrolytes and lipophilic properties of acetic acid can cause the reduction of intracellular pH, and the low intracellular pH will result in the release of DNA purines and pyrimidines and causes damage to the genome (Hahn et al. 1999; Van de Guchte et al., 2002). *Saccharomyces cerevisiae* could lead to chromosomal DNA breakdown into fragments when treated with acetic acid (Ribeiro et al. 2006). For microorganisms, DNA repair is a highly complex phenomenon, and one of the processes is nucleotide excision repair (NER) (Kuper and Kisker 2012; Van Houten et al. 2005). Several proteins, including excinuclease UvrA, B, C, D, and RecA, have been proven to be important for strain tolerance against acidic conditions (Grinholc et al. 2015; Sancar and Rupp 1983). Among them, UvrA, which belongs to the NER pathway, is involved in DNA repair of prokaryotic microorganisms and is the first induced protein of the NER mechanism in bacteria (Doolittle et al. 1986; Kuper and Kisker 2012; Van Houten et al. 2005; Verhoeven et al. 2002). In *Lactobacillus helveticus* UvrA contributed to acid and oxidative tolerance (Cappa et al. 2005).

In our previous research, the proteome of *A. pasteurianus* under the conditions of acetic acid being present and absent was analyzed (Zheng et al. 2017). Especially, we wish to find a probable extra mechanism for acetic acid tolerance of AAB besides the mechanisms mentioned above. Fortunately, the expression of excision repair protein UvrA was found improved by acetic acid. And then, the *uvrA* gene (APA01_RS07300) was knocked out and overexpressed in *A. pasteurianus* to study the effect on acetic acid tolerance and acetic acid fermentation collaboratively with other proteins responsible for acetic acid tolerance. Therefore, a rational strategy could be proposed to improve acetic acid fermentation.

Materials and methods

Strains and plasmids

A. pasteurianus AC2005 was registered in the Chinese General Microbiological Culture Collection Center (numbered CGMCC 3089). *Escherichia coli* JM109 was used for construction of recombinant vectors.

The plasmid pMD18-T (Takara) was used for cloning and sequencing analysis. Plasmid pMV24 (*Ap*^{r}, *lacZ*), given as a gift by the Mizkan Group Corporation, Japan, was used for overexpression of *uvrA* in *A. pasteurianus* (Fukaya et al. 1989). A suicide plasmid pSUP202 was used to construct the replacement vector pSUP202-*uvrA*::Km. *E. coli* HB101 containing plasmid pRK2013 was used as assistance to knockout the gene of *A. pasteurianus* AC2005 (Wei et al. 2014).

Media and culture conditions

Strains of *E. coli* were grown at 37 °C on Luria–Bertani (LB) medium. *A. pasteurianus* AC2005 was cultured in YPG medium (1% yeast extract, 1% peptone, and 3% glucose) at 30 °C. GYEA medium (3% glucose, 1.5% yeast extract, 3.5% ethanol, 2% $CaCO_3$, 1.7% agar) was used for DNA manipulation of *A. pasteurianus* AC2005.

GY medium (3% glucose, 1.5% yeast extract) and GYA medium (3% glucose, 1.5% yeast extract, and acetic acid were added to proper concentrations) were used for proteome assay and for analyzing acetic acid inhibition on cell growth. Acetic acid shock experiments were performed in GYA medium containing 4 or 6% acetic acid conditions to analyze the survival of *A. pasteurianus* under high acetic acid concentration stress.

The acetic acid fermentation was performed with GPAE medium (2% glucose, 2% peptone, 1% acetic acid, and 8% ethanol). The *A. pasteurianus* AC2005 seed medium was GYE medium (3% glucose, 1.5% yeast extract, and 3.5% ethanol). The acetic acid fermentation was implemented in a 5-L self-inspiriting fermenter (Nanjing Huike Bioengineering Equipment Corporation, Nanjing, China). The strains were grown for 24 h at 30 °C in the GYE medium and then were transferred into the GPAE medium with 10% inoculum and grown at 30 °C and 0.15 vvm.

uvrA disruption and overexpression in *A. pasteurianus*

The deletions of *uvrA* from the chromosome of *A. pasteurianus* AC2005 were performed using the reported strategy with minor modifications (Wei et al. 2014; Zhu et al. 2011). Two fragments for the homologous recombination and the kanamycin resistance gene were obtained by polymerase chain reaction (PCR) using the primers *uvrA*-1/2 and kan-1/2. The PCR products were digested and ligated to the suicide plasmid pSUP202, yielding the gene replacement vector pSUP202-*uvrA*::Km. It was then transferred into *A. pasteurianus* AC2005 by triparental mating using *E. coli* JM109 bearing the respective vector as the donor and *E. coli* HB101 bearing the plasmid pRK2013 as the helper strain. The positive strains can grow on YGEA medium containing kanamycin and produce a transparent zone by producing acetic acid. The gene disruption was confirmed by PCR using the primers of *uvrA*-1/2 and kan-3/4.

The gene *uvrA* with the promoter sequence was amplified using *A. pasteurianus* AC2005 genomic DNA as a template, and the primers *uvrA*-3/4 were used for PCR. The pMV24 plasmid was used for constructing the *uvrA* overexpression vector pMV24-*uvrA*. The control strain *A. pasteurianus* AC2005 (pMV24) and the overexpression strain *A. pasteurianus* AC2005 (pMV24-*uvrA*) were constructed by electroporation with the plasmid pMV24 and plasmid pMV24-*uvrA*, respectively. Primers used in this study are listed in Table 1. DNA manipulation was performed according to standard protocols (Sambrook and Russell 2016).

Detection of gene transcription

For quantitative real-time PCR (RT-PCR) experiments, strains were cultured at different acetic acid concentrations (0, 1, and 2%), and the cells were collected when the $OD_{610\ nm}$ (optical density under 610 nm) reached about 0.6.

Total RNA was isolated using RNAplus Kit (TaKaRa Biotechnology, Dalian, China) by following the manufacturer's procedure. Total RNA was treated with DNase I for 30 min at 37 °C to remove residual DNA. RNA samples were reverse-transcribed with RevertAid™ First Strand cDNA Synthesis Kit (Takara Biotechnology, Dalian, China) according to the manufacturer's instructions. Subsequently, the quantitative gene analysis was performed on an ABI StepOnePlus Real-Time PCR System (StepOnePlus, Applied Biosystems, USA) using the oligonucleotides listed in Table 1. The 16S rRNA gene was used as the internal standard. The ^{2}Ct method was applied to analyze the data.

Acetic acid tolerance analysis

The acetic acid tolerance of strains AC2005 (pMV24), AC2005-Δ*uvrA*, and AC2005 (pMV24-*uvrA*) that expressed UvrA in different levels was analyzed by comparing their OD at 610 nm after 48-h cultivation under different initial acetic acid concentrations with 10% inoculum.

To test the tolerance of the strains toward higher acetic acid stresses, shock experiments were performed. Strains were grown overnight at 30 °C in GY medium and then were diluted with fresh GY medium at a ratio of 1:100 to grow to a mid-exponential phase (reaching an $OD_{610\ mm}$ of 0.6). Acetic acid was added to final concentrations of 2, 4, and 6% (*v*/*v*). After 20 and 40 min of incubation at 30 °C, cells were added into an Oxford cup and grew on GYEA medium, and the survival of cells was observed. Viable bacteria were determined by spread plating serial dilutions onto the GYEA medium.

Genome integrality analysis

For genome damage analysis, overnight cultures of AC2005 (pMV24), AC2005-Δ*uvrA*, and AC2005 (pMV24-*uvrA*) were diluted 10-fold into fresh YPG medium and grown to mid-exponential phase (when the $OD_{610\ nm}$ reached about 0.6). Then, cells were harvested and resuspended into 10 mL of GY medium and GYA media with acetic acid concentrations (*v*/*v*) of 2, 4, and 6% to incubate for 40 min at 30 °C. Furthermore, genomic DNA was isolated with a TIANamp Bacteria DNA Kit (TIANGEN, Beijing, China). DNA strand

Table 1 Primers used in this study

Primer	Sequence(5′–3′)	Purpose
uvrA-1	TGCACTGCAGTCTGTTTCTCCTTATGCG	*uvrA* amplification
uvrA-2	CATGCCATGGTTAGAAAAACTCATCGAGCAT	*uvrA* amplification
Kan-1	CATGCCATGGGCGAGGTATGTAGGCGGTGCT	Km^r amplification
Kan-2	CATGCCATGGTTAGAAAAACTCATCGAGCAT	Km^r amplification
Kan-3	CGGAATTCATGAGCCATATTCAACGG	Km^r amplification
Kan-4	TGCTCTAGATTAGAAAAACTCATCGAGC	Km^r amplification
uvrA-3	CGGAATTCGGGGCCATTTTTATTGCG	*uvrA* amplification
uvrA-4	GCTCTAGATTATAGATATTCACTTAGAAAAGGC	*uvrA* amplification
16s-F	TCCTACGGGAGGCAGCAGT	RT-PCR
16s-R	CCTACACGCCCTTTACGC	RT-PCR
U-F	AATTAGACCGTGGAGTGCG	RT-PCR for *uvrA*
U-R	CAGAAAGAGCCTGTTGAGC	RT-PCR for *uvrA*
adh-F	CCAAAACGCACCTGGTCTAT	RT-PCR for *adh*
adh-R	TCTTCCAGACCGTTTCCATC	RT-PCR for *adh*
cs-F	TTTCACGTTTGACCCAGGTT	RT-PCR for *cs*
cs-R	GCAGCAGCGTATGGTTTGTAAG	RT-PCR for *cs*
dnaK-F	CCGTTCTGAAGGGTGATGTTA	RT-PCR for *dnak*
dnaK-R	TCGAAGTTACCCAGCAGCTT	RT-PCR for *dnak*

breaks were also demonstrated at the single-cell level by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay with the In Situ Cell Death Detection Kit, fluorescein, from Roche Molecular Biochemicals (Mannheim, Germany) (Gavrieli et al. 1992; Trcek et al. 2006). The integrality of the genomic DNA was analyzed by 1.0% agarose gel electrophoresis (40 V, 60 min) by comparing the trailing (Gavrieli et al. 1992; Poorbagher et al. 2016).

Effect of UvrA expression on acetic acid fermentation

To test the effect of UvrA on acetic acid fermentation, acetic acid fermentations were performed in a 5-L fermenter as mentioned above. The growth and the acetic acid were monitored, and the relative transcription of *uvrA*, *adh*, *cs*, and *dnaK* was mainly detected.

Analytical methods

The cell growth was monitored based on the OD value by a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) at 610 nm. The acidity of the broth was titrated by 0.1 M NaOH with phenolphthalein as an indicator. All experiments were performed in triplicate. The results were expressed as mean values with standard error. Analysis of the differences between the categories was calculated with a confidence interval of 95% with SPSS (least significant differences) analysis.

Results

Effect of acetic acid on cell growth and protein expression

The growths of *A. pasteurianus* AC2005 under different concentrations of acetic acid added at initial time were compared at 48 h. As shown in Fig. 1a, the cell growth reduced with the increase of acetic acid. When the initial acetic acid concentration was above 3%, the time for doubling of initial OD was more than 80 h (data not shown).

To further study the effect of acetic acid on cells, the differential protein expressions of *A. pasteurianus* AC2005 under 0 and 1% acetic acid concentrations were analyzed by using the proteome assay (Zheng et al. 2017). The major up-regulated proteins were related with energy production and conversion, amino acid transport and metabolism, carbohydrate transport and metabolism, and ribosomal structure (Zheng et al. 2017). That differential protein expression is consistent with previous reports (Wang et al. 2015; Xia et al. 2016). Interestingly, three of the proteins, excinuclease ABC subunit A (UvrA) (GI:258541116), NAD-dependent DNA ligase (NAD-DNA ligase) (GI:918718363), and DNA

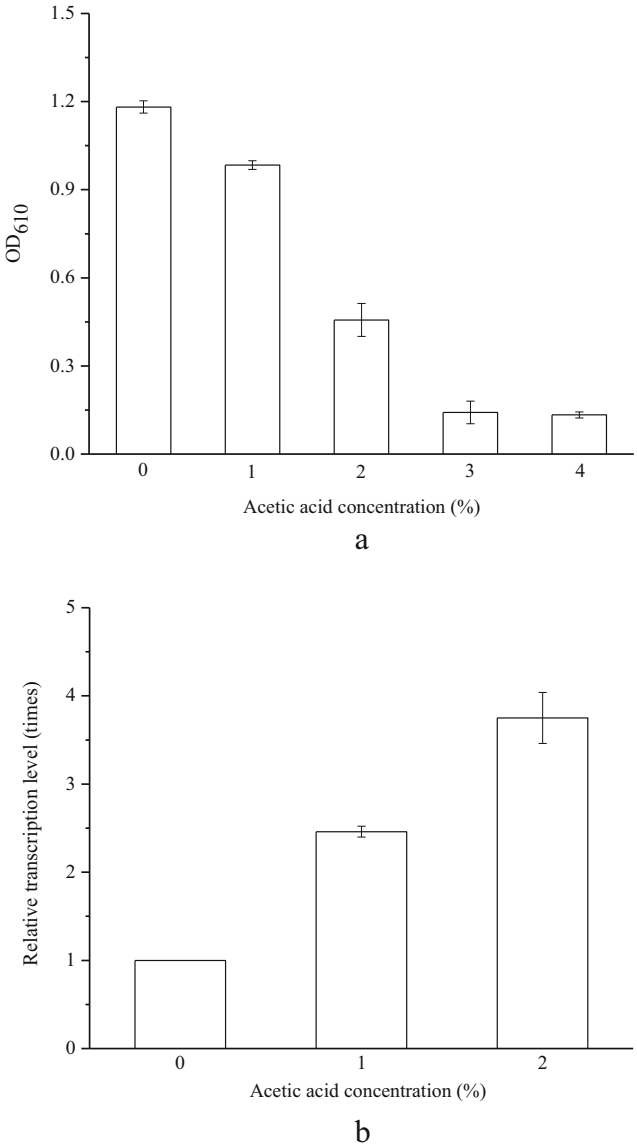

Fig. 1 Effect of acetic acid on cell growth and *uvrA* transcription. **a** Cell growth of the wild strain of *A. pasteurianus* AC2005. Cells were cultured in GY (0% acetic acid) and GYA medium (1, 2, 3, and 4% acetic acid), respectively, for 48 h. **b** Transcription levels of *uvrA* induced by acetic acid. The cells were harvested when $OD_{610\ nm}$ reached about 0.6

recombination/repair protein (RecA) (GI:256632226), involved in DNA recombination and repair were up-regulated when compared with those without acetic acid. RecA and NAD-DNA ligase were up-regulated 1.8 times and 1.5 times, respectively. Especially, UvrA was up-regulated 2.1 times. However, its relation with acetic acid tolerance of AAB was rarely studied. Thus, we analyzed the relative transcription of *uvrA* under acetic acid stress by RT-PCR. As shown in Fig. 1b, the relative transcriptions of *uvrA* increased with the increase of acetic acid concentrations. Furthermore, the analysis of genome integrality by agarose gel electrophoresis showed that the diffusion increased with the increase of acetic acid (the same as the Fig. 3b). Those results indicated that acetic acid

could affect the genome integrality, and some proteins related to DNA repair, such as UvrA, were up-regulated under acetic acid conditions, together with other proteins related to acetic acid tolerance.

Effect of UvrA on acetic acid tolerance and genomic DNA integrality

To analyze the effects of UvrA on *A. pasteurianus* acetic acid tolerance, three strains, *uvrA* knockout strain AC2005-Δ*uvrA*, *uvrA* overexpression strain AC2005 (pMV24-*uvrA*), and the control strain AC2005 (pMV24), were constructed according to the method described above. Strains were grown in GYA media supplemented with different acetic acid concentrations to compare the effect of UvrA on cell growth. As shown in Fig. 2a, all strains grew similarly at 0% ($P < 0.05$) without acetic acid press; however, the knockout strains hardly grew when the concentration of acetic acid was more than 1%. Whereas, the biomass of AC2005 (pMV24-*uvrA*) was 20.6 and 31.7% higher than that of the control under 1 and 2% acetic acid, respectively. The growth of the control and AC2005-Δ*uvrA* was almost suppressed by 3% acetic acid concentration even after 72 h of cultivation, but the biomass of AC2005 (pMV24-*uvrA*) increased by 66.1% (data not shown). Then, *uvrA* transcriptions were compared under acetic acid concentration of 0, 1, and 2%, since three stains hardly grow when the initial acetic acid concentration was more than 2%. As shown in Fig. 2b, *uvrA* transcription was positively correlated with the growth of strains under acetic acid conditions. Thus, strain AC2005 (pMV24-*uvrA*) showed a better cell growth under acetic acid stress than the control due to the overexpression of UvrA.

Furthermore, acetic acid shock experiments were performed to determine the effect of UvrA overexpression on the acetic acid tolerance. As shown in Fig. 3a, the viable cells decreased with the increase of acetic acid concentration and treatment period. The knockout strain hardly grew after the 40-min acetic acid shock and showed a clearly decreased tolerance to acetic acid than those of the control. The viable cells of UvrA overexpression strain are more than those of the control strain exposed in the same conditions. The decrease of survival ratio with the acetic acid concentration and treatment period reflected the toxicity of acetic acid to cells (shown in Fig. 3b). With 6% acetic acid shock for 20 and 40 min, the survival ratio of overexpression strain AC2005 (pMV24-*uvrA*) was 2 and 0.12%, respectively. However, it was 1.5 and 0.06% for the control strain AC2005 (pMV24).

The DNA could be damaged by acids to result in the breakage of a DNA strand and the decrease of the genome length. DNA damage analysis is an important method in screening chemicals and other factors for potential genotoxic and cytotoxic effects, which could be detected by agarose gel electrophoresis (Drouin et al. 1996). In this study, the TUNEL apoptosis detection kit (FITC) and the agarose gel electrophoresis were used to analyze the qualitative DNA damage caused by

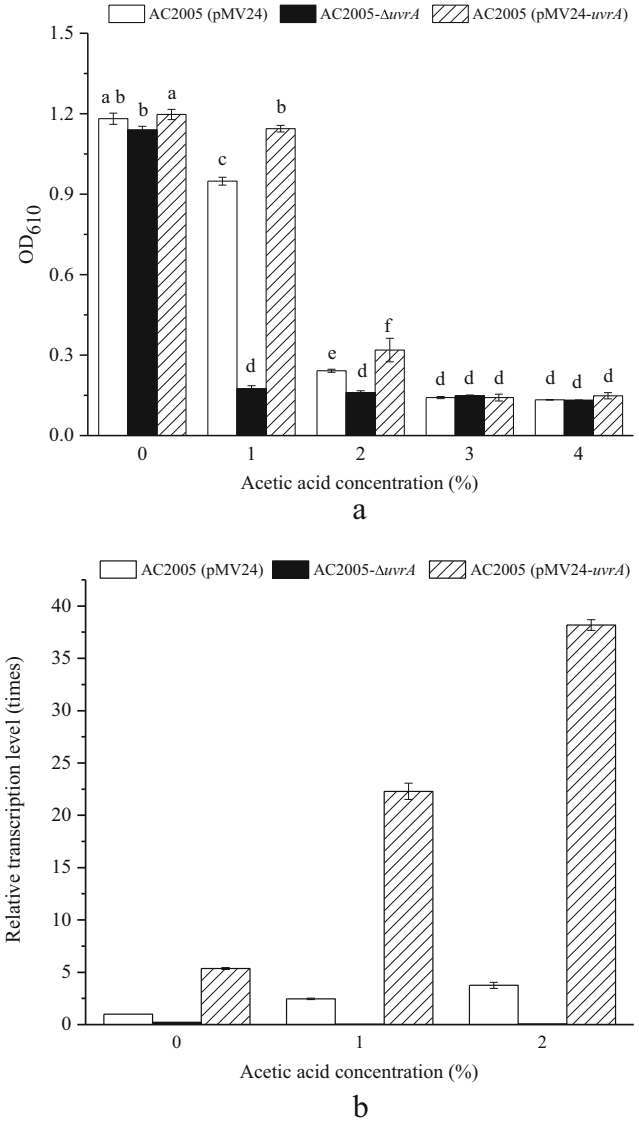

Fig. 2 Effect of UvrA expressions on cell growth. **a** Cell growth. Cells were cultured in GY and GYA media for 48 h. **b** Transcription of *uvrA*. The cells were harvested when $OD_{610\ nm}$ reached about 0.6. Cells of AC2005-Δ*uvrA* under 1 and 2% acetic acid conditions were collected after 24 h of cultivation since it hardly grew

acetic acid (Ribeiro et al. 2006). As shown in Fig. 4a, b, the number of FITC-positive cells that indicated the DNA damage caused by acetic acid and the diffusion of DNA samples increased with the increase of acetic acid concentrations. When the concentration of acetic acid is 6%, FITC-positive cells of AC2005 (pMV24), AC2005-Δ*uvrA*, and AC2005 (pMV24-*uvrA*) were 7.00-, 9.23-, and 4.30-folds higher than those without acetic acid, respectively. Clearly, UvrA overexpression would help to protect the integrality of the genome, and knockout of *uvrA* resulted in serious damage. Especially, strain AC2005 (pMV24-*uvrA*) showed an improved acetic acid tolerance and genome integrality because of the overexpression of UvrA with its own promoter, which was induced by acetic acid.

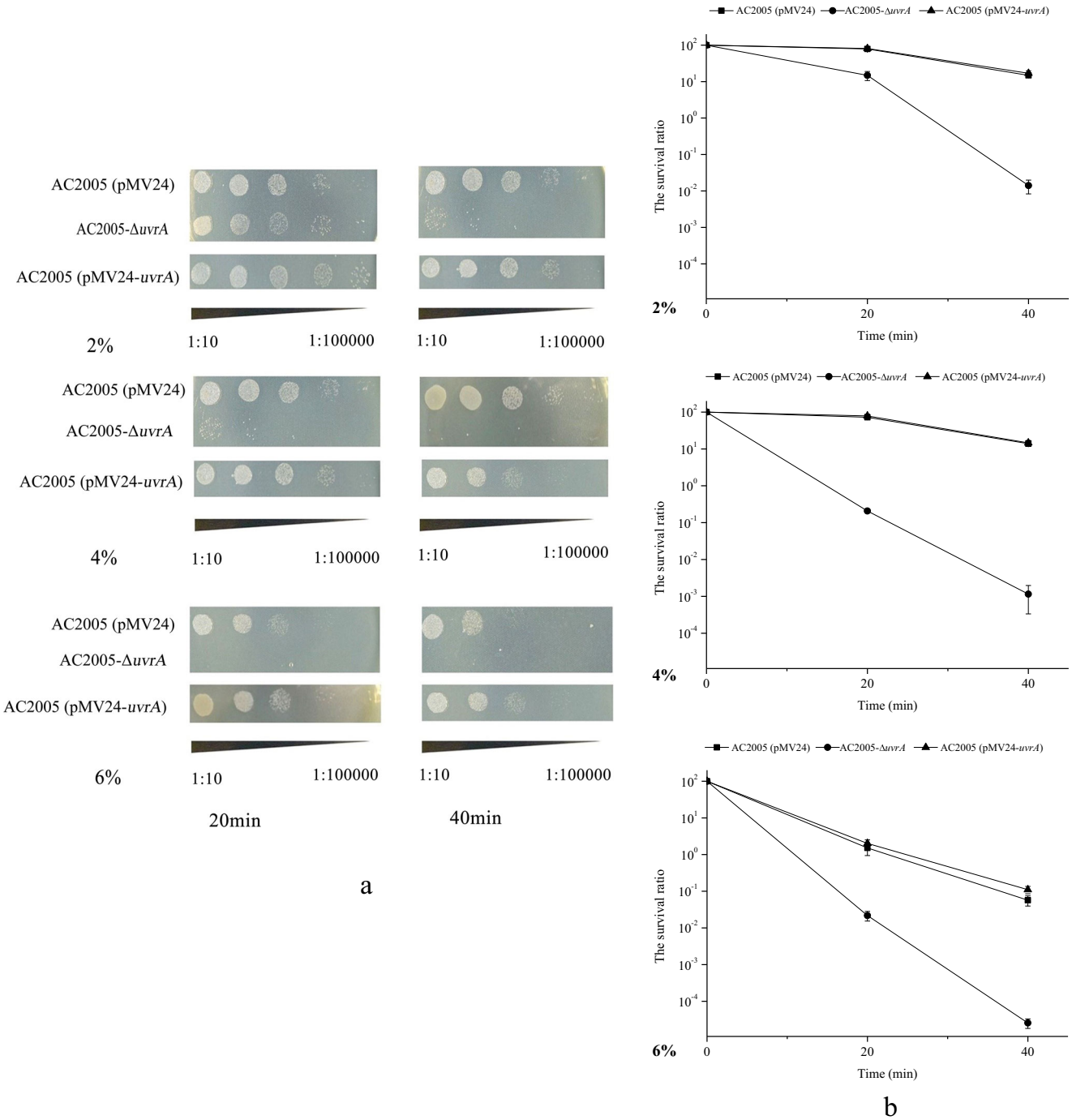

Fig. 3 Effect of UvrA expressions on acetic acid tolerance. **a** The bacterial colony after acetic acid shock of 2, 4, and 6%, respectively. The cells were treated with GYA media containing 2, 4, and 6% acetic acid, respectively, for 40 min. The dilution ratio from left to right is 1:10 to 1:10000. **b** The survival of strains after acetic acid shock of 2, 4, and 6%, respectively. The cells were treated with GYA media containing 2, 4, and 6% acetic acid, respectively, for 40 min. The survival ratio was calculated as the ratio of the viable cell count after and before treatment

All these results demonstrated that the overexpression of UvrA could increase the acetic acid tolerance of *A. pasteurianus* and knockout of *uvrA* will result in sensitivity to acetic acid. Briefly, acetic acid can cause DNA damage to inhibit *A. pasteurianus* growth, and UvrA that relates to the nucleotide excision repair process is responsible for acetic acid tolerance by protecting the genome from acetic acid damage.

Effect of UvrA on acetic acid fermentation

Acetic acid fermentations were performed with three strains. The time curves are shown in Fig. 5a. The cell growth and acetic acid production of knockout strain were almost inhibited due to the toxicity of 1% initial acetic acid concentration, while the growth and the acetic acid production of

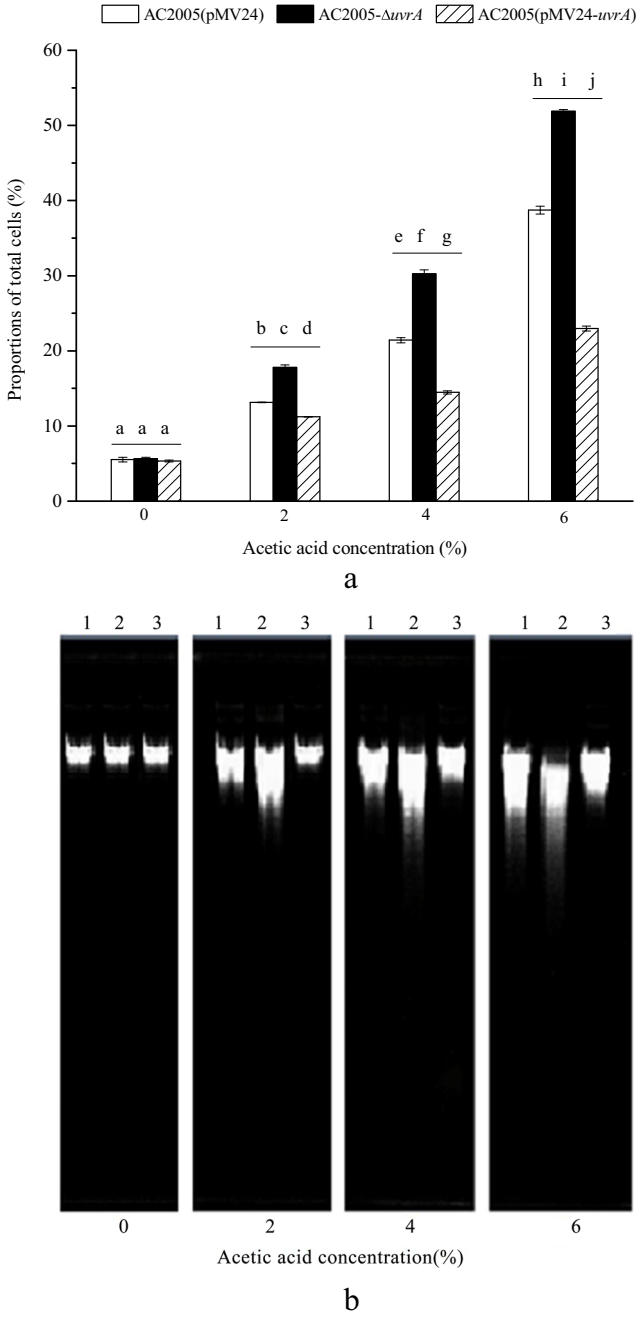

Fig. 4 Effect of UvrA expressions on genome integrality under acetic acid conditions. **a** TUNEL assay of FITC-positive cells. The cells were treated with GY medium and GYA media containing 2, 4, and 6% acetic acid, respectively, for 40 min. Letters present significantly different LSD tests at $P < 0.05$. **b** Agarose gel electrophoresis assay. 1, AC2005 (pMV24); 2, AC2005-Δ*uvrA*; 3, AC2005 (pMV24-*uvrA*). The cells were treated with GY medium and GYA media containing 2, 4, and 6% acetic acid, respectively, for 40 min

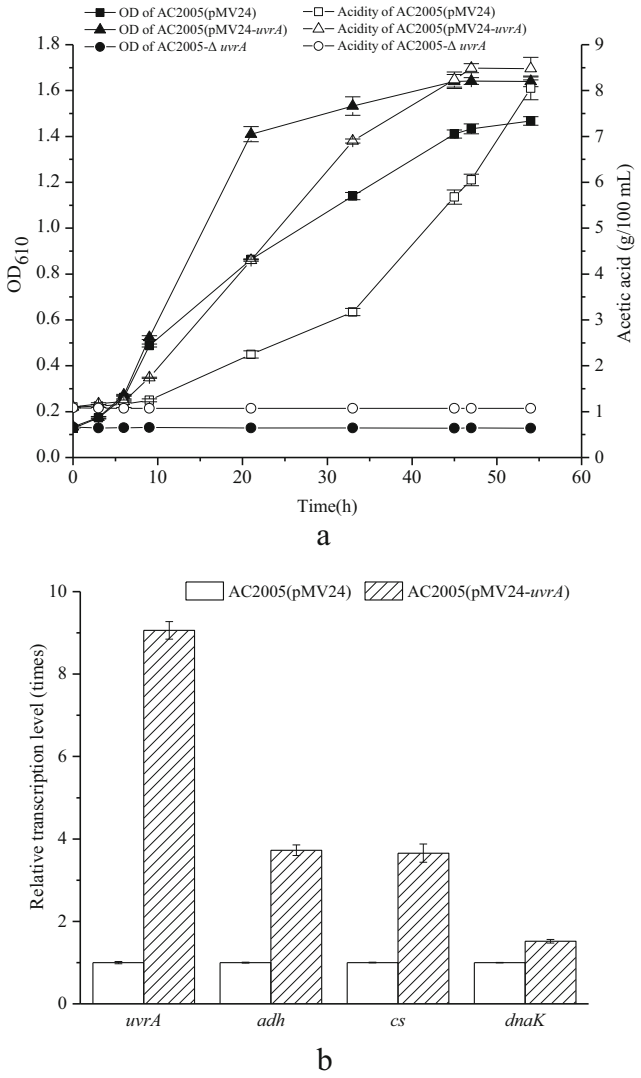

Fig. 5 Effect of UvrA expressions on acetic acid fermentation. **a** Time curves of acetic acid fermentation by strains with different UvrA expressions. **b** Transcription levels of genes related to acetic acid tolerance during fermentation. The cells were collected when the $OD_{610\ nm}$ reached about 0.6

overexpression strain were significantly higher than those of the control strain ($P < 0.05$) after 9 h. For AC2005 (pMV24-*uvrA*), the highest acidity (8.5 g/100 mL) was obtained at 47 h of fermentation that was shorter than the control strain by 7 h. The average acetification rate of AC2005 (pMV24-*uvrA*) was 1.57 g/(L h), which was 21.7% high when compared with the control (1.29 g/(L h)).

To confirm the reason for improved acetic acid fermentation, relative transcriptions of genes *adh*, *uvrA*, *cs*, and *dnaK* encoding the proteins ADH, UvrA, CS, and DnaK, respectively, which have been proved responsible for ethanol oxidation and acetic acid tolerance in AAB, were analyzed. As shown in Fig. 5b, the relative transcription of *uvrA*, *adh*, *cs*, and *dnaK* in AC2005 (pMV24-*uvrA*) were 9-, 3.7-, 3.6-, and 1.5-folds higher, respectively, than those of the control strain due to the overexpression of UvrA. Thus, UvrA not only improved the acetic acid tolerance of *A. pasteurianus* by reducing the genome damage caused by acetic acid, but also enhanced the

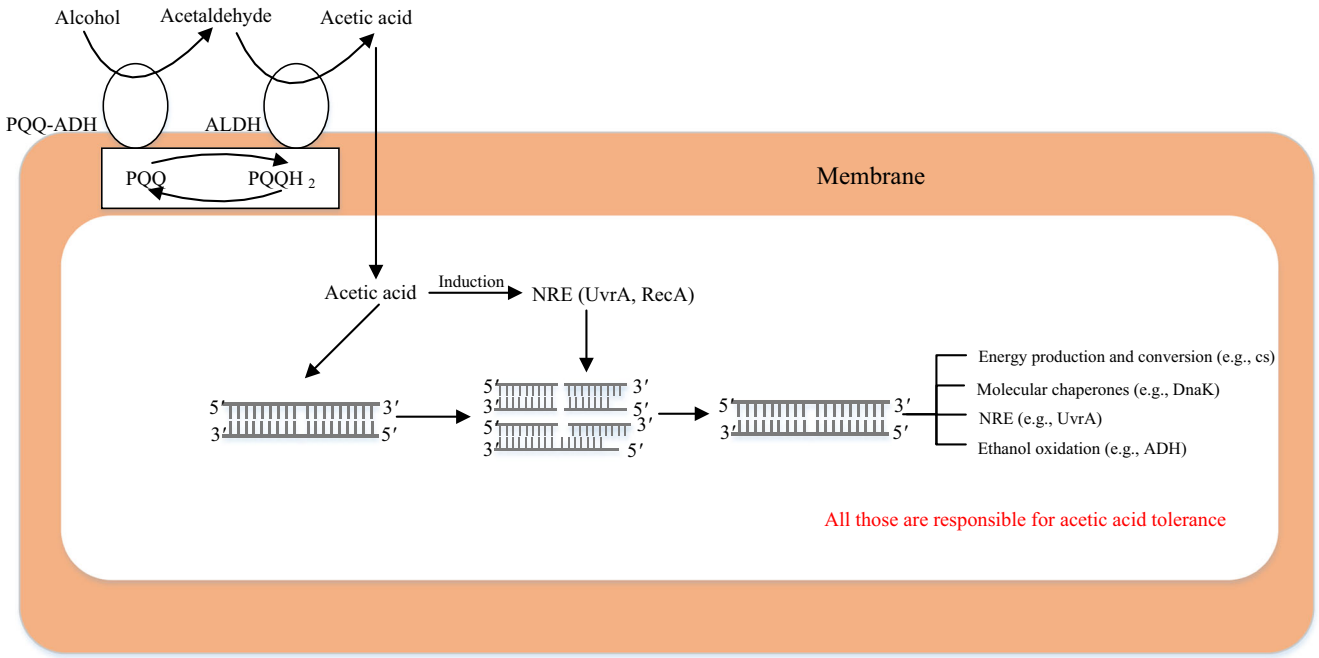

Fig. 6 Mechanism of *A. pasteurianus* acetic acid tolerance involving UvrA

expression of proteins related to ethanol oxidation and acetic acid tolerance to improve acetic acid fermentation.

Discussion

The destruction to bacteria by acetic acid is caused by intensifying the intracellular acidic environment and causing an uncoupling effect acting as a lipotrope (Yin et al. 2017). The remarkable acetic acid tolerance is an essential character to AAB, especially for acetic acid fermentation. The mechanism of this important property has been widely studied. However, few reports analyzed the acetic acid tolerance mechanism of AAB, considering the nucleotide repair. In general, the genome of all organisms is stable to ensure the metabolism and reproduction. Physical and chemical mutagens induce DNA lesions and reduce its molecular weight, besides stress conditions such as heat and peroxide (Brennan et al. 2000; Drouin et al. 1996; Greer and Zamenhof 1962). An intracellular acidic environment would cause the loss of more DNA purines and pyrimidines than the relative neutral environment (Cotter and Hill 2003). To resist these damages, some mechanisms are generated in the organism. The NER is one of the most common mechanisms to maintain the completeness of DNA. This repair system essentially repairs all DNA lesions and plays a backup role for other repair systems (De Laat et al. 1999; Sancar and Tang 1993). It is related to acid, heat, oxidation tolerances, etc. (Cappa et al. 2005; Yamamoto et al. 1996; Zheng et al. 2015). UvrA is the initial protein and is mainly involved in the original DNA damage detection and identification (Kuper and Kisker 2012; Van Houten et al. 2005). Furthermore, the overexpression of UvrA from *A. pasteurianus* could increase the tolerance of *E. coli* to acetic acid, heat, and peroxide (Zheng et al. 2015).

In this research, we demonstrated that acetic acid destroyed bacteria by affecting the genome integrality, and *A. pasteurianus* could reduce this negative effect on genomes by its DNA repair mechanism including nucleotide repair excinuclease UvrA. Moreover, the genome damage of *A. pasteurianus* increases with the increase of acetic acid accompanied with growth delay. *A. pasteurianus* could repair genome damage caused by the up-regulated expression of UvrA. In *A. pasteurianus*, UvrA was induced by acetic acid and the relative transcription level of *uvrA* increased with the increase of acetic acid to protect the genomic DNA by acetic acid. Overexpression of UvrA in *A. pasteurianus* protected the genomic DNA to a certain extent, while *uvrA* knockout resulted in the exacerbation of genome damage. A concentration of 1% acetic acid was almost lethal to *uvrA* knockout strain AC2005-Δ*uvrA*. As a result of the increase of genome integrality due to the overexpression of UvrA, some enzymes involved in ethanol oxidation, TCA cycle, and molecular chaperone were up-regulated to improve acetic acid fermentation (shown in Fig. 6).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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