Chapter 18
Expression of Gene *uvrA* from *Acetobacter pasteurianus* and Its Tolerance to Acetic Acid in *Escherichia coli*

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Abstract The *uvrA* gene of *Acetobacter pasteurianus* AC2005 coding for subunit A of the excinuclease ABC complex involved in the nucleotide excision repair mechanism was identified. Gene *uvrA* was amplified using *A. pasteurianus* AC2005 genomic DNA as a template. Then the pMV24 plasmid, an expression vector of *Acetobacter*, was used for constructing the recombinant plasmid pMV24-*uvrA*. UvrA was expressed in *Escherichia coli* JM109, and its molecular weight was about 91.1 kDa. With 0.5 % acetic acid shock for 20 and 40 min, the survival rates of recombinant strain *E. coli* JM109/pMV24-*uvrA* were 0.48 and 0.056 %, which increased by 17.5 and 10.2 times, respectively, compared with those of *E. coli* JM109/pMV24. All these demonstrate that the expression of repair excinuclease UvrA could increase the acetic acid tolerance of the strain.

Keywords *E. coli* · *uvrA* · Acetic acid · Gene expression

18.1 Introduction

Acetate ion in the microorganism is toxic mainly to itself, and acetobacter can improve its resistance to a high concentration of acetic acid with a unique mechanism. Studies suggest that there are two main mechanisms that confer high acetic acid concentration to the bacteria. One is to maintain the intracellular pH relatively constant, like the proton pump, overoxidation of acetic acid, changes in fatty acid

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composition in membrane, etc.; all these could affect the permeability of the proton [1, 2]. Another is to repair the damage of cellular components caused by high concentrations of acetic acid, like improvement of the stability of enzyme under high concentrations of acetic acid, correct folding of the protein involved by molecular chaperone, and so on [3–5].

Nucleotide excision repair (NER) is a mechanism commonly used to maintain the integrity of DNA and the proteins involved in this mechanism are mainly UvrA, UvrB, and UvrC [6]. UvrA is the initial induced protein in bacteria that test various structurally unrelated DNA lesions and excise and repair them. In prokaryotic microorganism, repair excinuclease UvrA involves in the excision and repair of DNA, and this repair mechanism is applicable to repair of many DNA damages [7]. According to the literature, in *Thermus thermophilus*, UvrA was overexpressed induced by IPTG and it makes the strain resist high temperature at neutral pH and resist low pH at room temperature. In *Lactobacillus helveticus*, UvrA was activated by exposure to UV radiation and oxidative stress, and the expression of *uvrA* was inducible by pH; UvrA contributes to acid and oxidative tolerance in *L. helveticus* [8].

Acetobacter pasteurianus is an important acid-producing bacterium during solid-state fermentation of vinegar, and is one of the bacteria commonly used in pure liquid fermentation worldwide. As its important application value, the whole-genome of *A. pasteurianus* has been sequenced completely [9, 10]. This research is focused on the repair excinuclease UvrA from *A. pasteurianus* AC2005. Using genetic engineering technology, we study the effects of UvrA on the acetic acid tolerance of *Escherichia coli*, which would lay the foundation for further clarifying the function of repair excinuclease.

18.2 Materials and Methods

18.2.1 Bacterial Strains, Plasmids, and Growth Conditions

Acetobacter pasteurianus AC2005, stored in the laboratory, was cultured in GYE media (2 % glucose, 1.5 % yeast extract, 3.5 % ethanol) at 30 °C. *E. coli* JM109 were used as hosts for the cloning experiments and *E. coli* was grown at 37 °C on Luria–Bertani (LB) broth supplemented with or without 100 μg/ml ampicillin. The pMD19-T simple vector (TaKaRa) was used for both cloning and sequencing analysis. The pMV24 plasmid (Ap^r, lacZ), gifted by Mizkan Group Corporation, Japan, was used for expression of the gene in *E. coli*.

18.2.2 Construction of the Recombinant Plasmid and Expression of the Target Protein

We used expression vector pMV24 (Ap^r, 3,854 bp) and selected two restriction sites (*Eco*RI and *Xba*I) to construct recombinant plasmid. As pMV24 had a lactose promoter, the target protein was expressed induced by IPTG.

18.2.3 Shock Experiments

To test the tolerance of the strains toward extremely high acetate stresses, shock experiments were performed with much higher concentrations of acetic acid. Strains were grown overnight at 37 °C in LB medium containing 100 mg/mL ampicillin, then diluted at a ratio of 1:100 into fresh LB medium containing 100 mg/mL ampicillin, and grown at 37 °C to logarithmic phase reaching an OD_{600} value of 0.6, then added 1 mM IPTG and inoculated for 4–5 h to induce the expression of UvrA. After that acetic acid was added to final concentrations of 0.5 % (v/v). With 40 min incubation at 37 °C, samples were removed and the number of viable bacteria was determined by spread plating serial dilutions onto LB agar containing 100 mg/mL ampicillin. The plates were incubated at 37 °C for about 16 h before enumeration of the colonies. At the same time, the cultures shocked for 20 and 40 min were serially diluted, plated onto LB/agar plates with the Oxford Cup, and incubated at 37 °C for 16 h and then photographed.

18.3 Results

18.3.1 Sequence Analysis of the uvrA in A. pasteurianus AC2005

The *uvrA* gene of *A. pasteurianus* AC2005 was cloned and its nucleotide sequence was determined. Analysis of the sequence revealed a gene of 2,514 nt that encoded a protein with 837 amino acids (aa) and a predicted molecular mass of 91.1 kDa. In this study, UvrA was expressed in *E. coli* JM109 induced by 1 mM IPTG. By SDS-PAGE, as shown in Fig. 18.1, a significant band of the protein induced appeared at about 91.1 kDa, and its molecular weight was in line with expectation.

The result of comparison indicates that the protein has high homology with the genus *Acetobacter* and *Gluconobacter*. The protein sequence contains a conserved region of NER enzymes.

The sequence obtained was compared with the *uvrA* gene sequence (GenBank: 8435212) of *A. pasteurianus* IFO3283-01 in GenBank. The result showed that the coding sequence from *A. pasteurianus* AC2005 has a similarity of 93.2 % with the

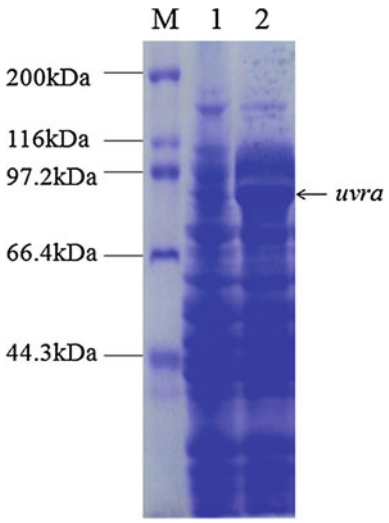

Fig. 18.1 Expression of pMV24-*uvrA* induced by IPTG *M* Marker; *1 E. coli* JM109/pMV24; *2 E. coli* JM109/pMV24-*uvrA*

corresponding region from *A. pasteurianus* IFO3283-01. Compared with the protein UvrA from *A. pasteurianus* AC2005 speculated by the coding region with UvrA from Acetobacter IFO3283-01, the amino acid sequence has a similarity of 98.6 %. Therefore, we can determine the amplified bands for *uvrA* gene of *A. pasteurianus*.

18.3.2 Effect of uvrA Expression on the Growth of E. coli Under Acidic Conditions

To determine the effect on cell growth caused by the expression of UvrA, the experiment studies the growth curves of the strains with the same initial amount of bacteria cells in LB medium. As acetic acid treatment could significantly reduce the final biomass of different strains, *E. coli* JM109/pMV24 and *E. coli* JM109/pMV24-*uvrA* were grown in LB broth supplemented with or without 0.05 % (vol/vol) acetic acid and their growth curves were determined. As shown in Fig. 18.2, in the presence of acetic acid, the growth of control strain *E. coli* JM109/pMV24 in early logarithmic phase and steady growth was suppressed obviously. However, the growth of recombinant strain *E. coli* JM109/pMV24-*uvrA* in the presence or absence of acetic acid is similar. It showed that the expression of UvrA increased the acetic acid tolerance of recombinant strain, and it initially proved that UvrA was related to the acetic acid tolerance.

As we all know acetic acid is highly toxic to *E. coli* cells, and the concentration of acetic acid for use in *E. coli* is often under 0.1 %. Then acetic acid shock experiments were subsequently performed to test the tolerance of *uvrA* expression strain toward acetic acid at higher concentration. The final concentrations of acid required to adjust cultures of *E. coli* to 0.5 % acetic acid were pH 3. After shocking

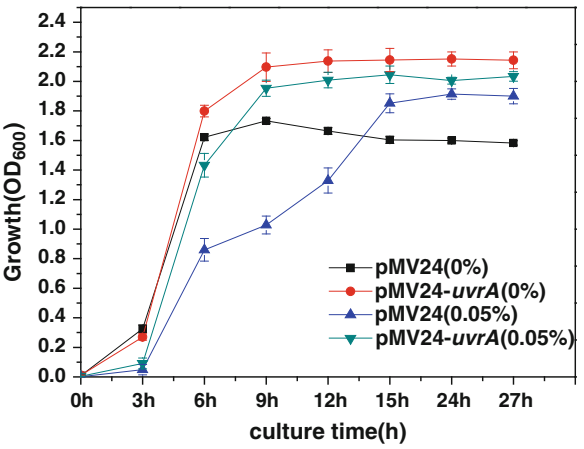

Fig. 18.2 Growth curves of *E. coli* JM109/pMV24 and *E. coli* JM109/pMV24-*uvrA* induced by IPTG with or without 0.05 % acetic acid, respectively

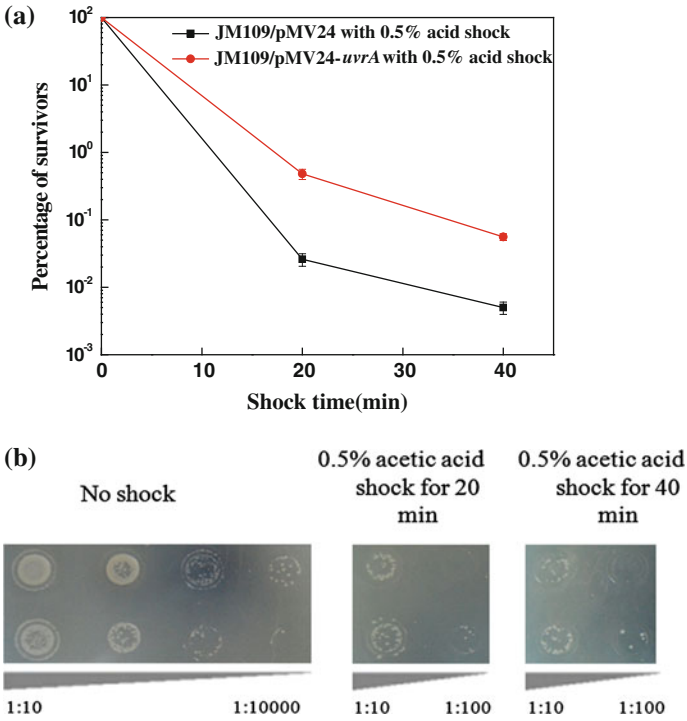

Fig. 18.3 Survival of *E. coli* JM109/pMV24 and *E. coli* JM109/pMV24-*uvrA* under acid-shock conditions, **a** bacterial survival rate at different times treated with 0.5 % acetic acid, **b** colony morphology shocked with 0.5 % acetic acid for 40 min

with 0.5 % acetic acid for different times, the survival curves for the control strain *E. coli* JM109/pMV24 and the recombinant strain *E. coli* JM109/pMV24-*uvrA* are as shown in Fig. 18.3. The changeable survival rate after the acetic acid treatment reflected that the expression of UvrA affects acetic acid tolerance of *E. coli*. With increasing shock time, the recombinant strain showed a clearly increased tolerance to acetic acid as compared to the control strain exposed to 0.5 % acetic acid. With 0.5 % acetic acid shock for 20 and 40 min, the survival rates of recombinant strain *E. coli* JM109/pMV24-*uvrA* were 0.48 and 0.056 %, which increased by 17.5 and 10.2 times, respectively, compared with those of control strain *E. coli* JM109/pMV24. And with 0.5 % acetic acid shock for 40 min, the plaques of two strains on solid plate were observed. Although the number of colonies of control strain under normal culture condition was slightly higher than the recombinant strain, the number was 10 times higher than control strain after acetic acid shock. All these demonstrate that the expression of repair excinuclease UvrA could increase the acetic acid tolerance of the strain.

18.4 Discussion

We have constructed a recombinant strain of *E. coli* with the help of the pMV24 plasmid which is an expression vector of *Acetobacter*. UvrA was expressed in *E. coli* JM109, and its molecular weight was about 91.1 kDa. Homology analysis of the *A. pasteurianus uvrA* gene product revealed high level homology to the *Acetobacter* and *Gluconobacter* UvrA proteins. The protein sequence contained a conserved region of NER enzymes was found. Comparison of the *A. pasteurianus* AC2005 and *A. pasteurianus* IFO3283-01 UvrA protein demonstrated 98.3 % identity and 98.6 % similarity.

With 0.5 % acetic acid shock for 20 and 40 min, the survival rates of recombinant strain *E. coli* JM109/pMV24-*uvrA* increased by 17.5 and 10.2 times, respectively, compared with those of *E. coli* JM109/pMV24. These demonstrate that the expression of repair excinuclease UvrA could increase the acetic acid tolerance of the strain.

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