Ultrasonics Sonochemistry 92 (2023) 106270

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Studies on ultrasound-mediated insertion-deletion polymorphisms of DNA and underlying mechanisms based on Ames tester strains

Yunliang Li [a,c], Siyu Ruan [b,*], Feng Lu [a], Pengfei Xie [a], Xiaoshuang Liu [a], Haile Ma [a,c,*]

[a] School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, PR China
[b] College of Tea and Food Science Technology, Jiangsu Polytechnic College of Agriculture and Forestry, 19 Wenchangdong Road, Jurong, Jiangsu 212400, PR China
[c] Institute of Food Physical Processing, Jiangsu University, 301 Xuefu Road, Zhenjiang, Jiangsu 212013, China

ARTICLE INFO

Keywords:
Ultrasound
Mutagenesis
InDels
Direct repeat sequences
Mechanism

ABSTRACT

Low-lethality ultrasound technology has received more and more attention in regulating microorganisms of fermentation industry. Herein, two representative Ames tester strains TA97a and TA98 as model organisms were used to explore the effects of ultrasound on insertion-deletion (InDel) polymorphisms of microbial DNA and its underlying mechanisms. Results revealed that a promotion was observed in the reversion mutation of TA98 upon sonication. Sequencing results from 1752 TA98 revertants showed that there was a total of 127 InDels, of which the InDels unique to ultrasound were 36 more than that of the control. Compared with the control, ultrasound-mediated InDels of DNA displayed additional −29 bp deletion and +7 ~ +43 bp insertions of direct repeat sequences. Combined with the analysis of transcriptomics and prediction of secondary structure of single-stranded DNA from InDels core region (No. 832 ~ 915 bp) in *hisD3052* gene of TA98 strain, ultrasound-mediated "thermal breathing" mechanism was proposed based on the formation of DNA hairpin structure with micro-homologous sequence. This finding implied that low-intensity ultrasound is expected to be developed a new low-lethal mutagenic technology for continuous mutagenesis.

1. Introduction

In recent years, environmentally-friendly low-intensity ultrasound is widely used in microbial industry due to its low lethality and great progress in the regulatory effect on microbial cells, such as microbial breeding or yield improvement [1–5]. These phenotypic feedbacks may be ascribed to the ultrasonic regulation effects on genes involved in diverse cellular functions [3]. Gene variation of DNA molecule mainly included single nucleotide variants (SNVs), large structural variants and small insertion-deletion (InDel), which is a common and functionally important type of sequence polymorphism and has been implicated in a number of diseases [6,7]. The InDel is a type of genetic variation in which a specific nucleotide sequence is present (insertion) or absent (deletion). Luo *et al.* reported that the high pressure produced by the electric field in the center of cavitation nucleus was enough to cause InDels in some genes of DNA molecule [8]. However, limited information on the research of InDel polymorphisms of DNA induced by ultrasound at the molecular level is available.

The Ames assay (*Salmonella* test), a widely accepted short-term bacterial assay, is often performed *in vitro* to evaluate potential DNA mutagenic effects caused by chemical compounds or physical factors [9–11]. The *Salmonella* strains in the Ames test were the preexisting mutations (such as TA97, TA98, TA100, TA102, TA104 and TA1535) with defective genes that prevent them from synthesizing the required amino acid called histidine, resulting in its incapable of growing and forming colonies in histidine absence. The reversion of defective genes mainly consisted of base substitution and frameshift mutation, which referred to the InDels in the site of defective genes. At present, the observed phenotypic reversion mutation of the frameshift representative Ames tester strain is usually supposed to be the classical InDels of wild-typed *his* gene. On some occasions, InDels of many non-core DNA sequences in protein structure will not significantly affect protein function [12,13]. Actually, the phenotypic reversion mutation caused by InDels in *his* gene of Ames tester strain includes three levels of reversion mutation in DNA sequence, protein sequence and protein function, respectively. In other words, gene changes not only refer in particular to the classical reversion to the wild-typed gene sequence (classical InDel) of DNA molecule, but also refer to some nonclassical InDels causing the restoration of protein function, thus leading to the phenotypic reversion mutation [14]. Therefore, systematic analysis of ultrasound-mediated

* Corresponding authors at: School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, PR China (H. Ma).
E-mail addresses: siyuruan@126.com (S. Ruan), mhl@ujs.edu.cn (H. Ma).

https://doi.org/10.1016/j.ultsonch.2022.106270
Received 10 October 2022; Received in revised form 5 December 2022; Accepted 14 December 2022
Available online 17 December 2022

InDels polymorphisms of DNA that can restore protein function using Ames tester strains will be conducive to broadening the application scope of ultrasound mutagenesis technology.

It is a classic research strategy to apply the model organisms with clear genetic background to reveal some universal life phenomena and mechanisms. In this study, two frameshift representative Ames tester strains of TA97a and TA98 (defective *hisD* genes that prevent them from synthesizing histidine) were selected as model microorganisms and effects of low-intensity ultrasound on their cell growth (total colonies) and reversion mutation were investigated. In addition, sequencing of revertants from strains with remarkable response to ultrasound were analyzed to explore ultrasound-mediated InDel polymorphisms of DNA. Combined with the analysis of transcriptomics and prediction of secondary structure of single-stranded DNA from InDels core region (No. 832 ~ 915 bp) in *hisD3052* gene of TA98 strain, ultrasound-mediated "thermal breathing" mechanism was proposed based on the formation of DNA hairpin structure with micro-homologous sequence. It is believed that this study will provide a theoretical foundation for the further development of low-lethality ultrasound mutagenesis technology in regulating microorganisms.

2. Materials and methods

2.1. Microorganism, maintenance and preparation

Salmonella typhimurium TA97a (*hisD6610*, *hisO1242*, *uvrB-bio*, *rfa, pKM101*), and TA98 (*hisD3052*, *uvrB-bio*, *rfa*, *pKM101*) were purchased from Moltox (Molecular Toxicology Inc., North Carolina, USA) as lyophilized and stablilized cell cultures, which were histidine dependent by virtue of a mutation in the histidine operon. The strains were revived and then stored at −80 °C according to the directions supplied by the Ames laboratory.

Ames tester strains (TA97a and TA98) were grown on broth solid medium (10.0 g/L tryptone, 5.0 g/L yeast extract, 5.0 g/L NaCl, 2.6 g/L $K_2HPO_4{\cdot}3H_2O$ and 20.0 g/L agar) at 37 °C for 24 h. A ring of strain from solid medium was cultivated in broth liquid medium (10.0 g/L tryptone, 5.0 g/L yeast extract, 5.0 g/L NaCl and 2.6 g/L $K_2HPO_4{\cdot}3H_2O$) at 37 °C with a shaking of 100 r/min for 24 h to reach $10^8 \sim 10^9$ colony forming units (CFU) /mL, which was employed as the seed liquid.

2.2. Growth curve

After inoculation with a 2% ratio of above seed liquid strain (Section 2.1) into sterilized broth liquid medium, the suspension was incubated at 37 °C under 100 r/min and absorbance values were determined at 600 nm at intervals of 2 ~ 4 h by spectrophotometry (TU-1800, Persee General Co., Ltd., Beijing, China).

2.3. Optimization of ultrasonic parameters for a maximum reversion mutation

2.3.1. Preparation of screening culture medium

The medium was prepared by the method [15] with some modifications. A aliquot of 8 mL phosphate buffer solution (175 g/L $NaNH_4HPO_4{\cdot}3H_2O$, 100 g/L $C_6H_8O_7{\cdot}H_2O$, 500 g/L K_2HPO_4 and 10 g/L $MgSO_4{\cdot}7H_2O$), 20 mL glucose solution (40%, w/v) and 0.6 mL histidine-biotin solution (0.5 mmol/L) were successively added into 400 mL sterilized agar medium (1.5%, w/v). Then, the solution was evenly mixed for preparing plates (7.5 cm in diameter), followed by condensation and solidification. Finally, the inverted plates were placed in the clean bench for further use.

2.3.2. Strains to receive ultrasound irradiation

The experimental protocol applied was exactly as described by the Ames laboratory [15] with slight modifications. The bacteria liquid of 2% was inoculated into the sterilized broth liquid medium, followed by incubation for a certain period at 37 °C and 100 r/min. Then, the suspension sample in a sealed conical flask was placed in the ultrasonic treatment tank to receive irradiation. The self-developed ultrasonic device (Fig. 1) was applied to stimulate the Ames tester strains. Influences of ultrasound time of 0, 5, 10 and 15 min (power density of 20 W/L and frequency of 40 kHz after incubation for 6 h), incubation for 4, 6 and 8 h (power density of 20 W/L and frequency of 40 kHz under the optimal ultrasonic time condition), power density of 10, 20, 40 and 60 W/L (frequency of 40 kHz under the optimal ultrasonic time and cultivation condition) and frequency of 20, 35, 40, 50 and 60 kHz (under the optimal ultrasonic time, power density and cultivation condition) on the revertant colonies and total colonies of Ames tester strains were investigated. The ultrasonic pulsed model was set at on-time for 4 s and off-time for 1 s. Meanwhile, the suspension without ultrasonic treatment was set as the control group. After sonication, the bacterial suspension was placed in incubator at 37 °C for 40 min. The number of revertants induced by sonication and the spontaneous revertants (without sonication) on screening culture medium was respectively counted and the reversion mutation rate of Ames tester strain was calculated combined with the total number of colonies (total CFU/mL) on broth medium plates. The ratio of the mutation rate of sonication group to the spontaneous mutation rate, namely the relative mutation multiple, was set as an index for evaluating ultrasound-mediated DNA mutation.

2.3.3. Colony counting of Ames tester strains

After incubation for 40 min, 75 μL of cell suspension was added into the plate of screening culture medium. Plates were placed at 37 °C for 48 ~ 60 h and the number of revertants was determined via plate colony counting. In addition, the sonicated cell suspension solution was serially diluted (approximately 10^{-6}) in sterilized water and 100 μL was added into the plate of broth solid medium, which was incubated at 37 °C for 24 h and colonies formed were counted as total CFU/mL. A preliminary estimation of each treatment was performed to determine the best range of colonies for counting. The reversion mutation rate and relative mutation (multiple) were determined as follows:

$$\text{Reversion mutation rate} = \frac{\text{Number of revertant CFU/mL}}{\text{Number of total CFU/mL}} \quad (1)$$

$$\text{Relative mutation (multiple)} = \frac{A_1}{A_0} \quad (2)$$

where A_1 is the reversion mutation rate of sonicated samples; A_0 is the reversion mutation rate of samples without sonication (spontaneous revertants).

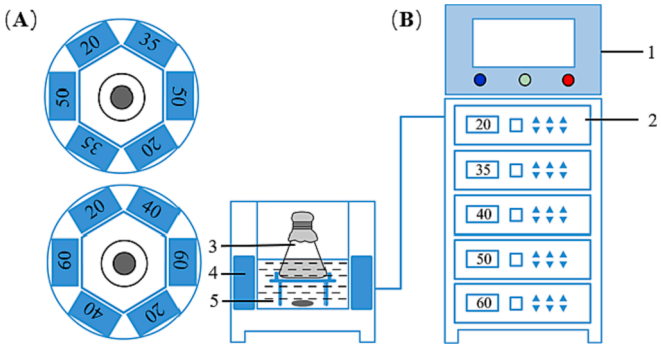

Figure 1. Divergent multi-frequency ultrasonic equipment including overhead view of transducer (A) and front view of ultrasound device (B). 1-electric control cabinet; 2-ultrasonic generator; 3-sample vessel; 4-ultrasonic transducer; 5-thermostatic water bath. Numbers on the ultrasonic transducer (overhead view) represent ultrasonic frequency of 20, 35, 40, 50 and 60 kHz, respectively, which are distributed symmetrically in a hexagon.

2.4. PCR amplification and sequencing of revertants

Under the optimal sonication condition, the revertant colonies (Section 2.3.3) with InDels were picked up and cultured in 500 μL screening liquid medium (2% phosphate buffer solution, 5% glucose solution (40%, w/v) and trace histidine-biotin solution (0.5 mmol/L) in sterilized water) for 12 h, which was used for further polymerase chain reaction (PCR) by PCR instrument (T100^{TM} Thermal Cycler, Bio-Rad, Shanghai, China). the primers (Primer 1 and Primer 2) were shown in Table 1 and the PCR program included pre-denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 30 s and extension at 72 °C for 90 s. Final extension was given at 72 °C for 10 min. The obtained amplified products were verified by polyacrylamide gel electrophoresis (PAGE) and sequencing was performed (Sangon Biotech Co., Ltd. Shanghai, China). Molecular evolutionary genetic analysis program (MEGA v7.0) was used for multiple sequence alignment analysis of sequencing results.

Based on the sequencing results (Primer 1 and Primer 2) of *hisD3052* gene of 1752 TA98 revertants (performed in triplicate), it was found that the InDels in *hisD3052* gene was concentrated in a short DNA region. In order to more intuitively and effectively distinguish the InDels in *hisD3052* gene by PAGE, the primers (Primer 3 and Primer 4, Table 1) of *hisD3052* were redesigned for PCR and the amplified products (372 bp) were also verified by PAGE for preliminary screening. Data points of total 1752 TA98 revertants were sequenced for statistical analysis of the distribution of ultrasound-mediated InDels in this study.

2.5. Polyacrylamide gel electrophoresis

PAGE is a method of separating DNA fragments and proteins based on size, structure and molecular weight [16]. In this study, non-denaturing or native PAGE was applied to verify the InDels in *hisD3052* gene of TA98 revertants. Briefly, the amplified products of *hisD3052* from TA98 revertants and TA98 strain (internal standard) were mixed thoroughly at the ratio of 7:3, which was used for the loaded sample. Subsequently, the separation was performed using an electrophoresis power supply set at 220 V/gel for 90 min using DYCZ-30C electrophoresis system (Liuyi Biotechnology Co., Ltd, Beijing, China). Finally, developing solution (8% glucose, 0.7% boric acid and 1.1% sodium hydroxide) was added into the gel in the TS-1000 decolorizing orbital shaker (Kylin-Bell Lab Instruments Co., Ltd, Jiangsu, China) with a speed of 60 r/min until clear electrophoretic bands appeared.

2.6. Analysis of transcriptomics

The Ames tester strain TA98 was sonicated under the optimum parameters according to 2.3.2. The TA98 strain without ultrasound treatment was set as the control. Subsequently, the bacterial cells were immediately placed in a constant temperature incubator at 37 °C for 0 min/10 min and then centrifuged at 4000 r/min for 15 min. The obtained bacterial precipitation (reverse mutant strains) was washed with sterilized distilled water repetitiously to remove the residual medium. Afterwards, the cell was quickly placed in liquid nitrogen for 2 min and stored at −80°C. All experiments were performed in triplicate. The nucleic acid extraction and data analysis were performed by Shanghai Rongxiang Biotechnology Co., Ltd. (Shanghai, China).

Table 1
Primers used for amplification of *hisD3052* gene in TA98 strain.

Primers	Sequence of primers (5́ → 3́)
Primer 1	ATGGAGTAAAGACCATGAGCTTCA
Primer 2	TGCTTGCTCCTTGAGGGCGT
Primer 3	GTCAGGTCAGCCAGCGTCT
Primer 4	GTAATCGCATCCACCAAATC

2.7. Statistical analyses

Each test was replicated three times unless stated otherwise. Data were expressed as means ± SEM. Results were analyzed by using one-way ANOVA and LSD test at $p < 0.05$ using SPSS v16.0 (IBM Corporation, USA). Graphs were drawn by OriginPro 2019b software (OriginLab, USA). The secondary structure of single-stranded DNA hairpin was predicted and drawn using the ssDNA structure analysis tools (http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/Predict1.html).

3. Results and discussion

3.1. Growth curves of Ames tester strains

The growth curves of Ames tester strains TA97a and TA98 were exhibited in Fig. 2, which could be as baseline data for identifying different growth phases. It was concluded that the latent phase was within 1 h, followed by an exponential period and stationary phases which began from 1 h and 14 h, respectively. In the metaphase of logarithmic growth period, due to the mass propagation of microorganisms and DNA rapid replication, *Salmonella* strains of 4 ~ 8 h were selected for further reversion mutation experiment.

3.2. Effect of ultrasound irradiation on reversion mutation of Ames tester strains

As seen in Fig. 3, the influences of ultrasonic time (duration of 0, 5, 10 and 15 min) on relative mutation multiple (reversion mutation rate of $10^{-6} \sim 10^{-9}$) and total CFU/mL of TA97a and TA98 were investigated at 20 W/L and 40 kHz after cultivation for 6 h. The results showed that there was no significant difference observed in the reversion mutation rate of TA97a between sonication group ($8.0 \sim 8.9 \times 10^{-8}$) and the control ($8.0 \times 10^{-8}$). In terms of TA98 strain, the maximum multiple (2.68) of relative mutation was achieved at sonication for 5 min, the reversion mutation rate of which was up to 1.1×10^{-6} (Fig. 3B). This suggested that sonication with an appropriately selected application regimen could promote the DNA mutation of TA98 strain. Despite of two strains belonging to frameshift mutations, the influence of ultrasound on reversion mutation of TA98 strain was greater than those of TA97a strain. This was similar to previous studies which displayed different mutagenic responses of TA97a and TA98 to a range of reference

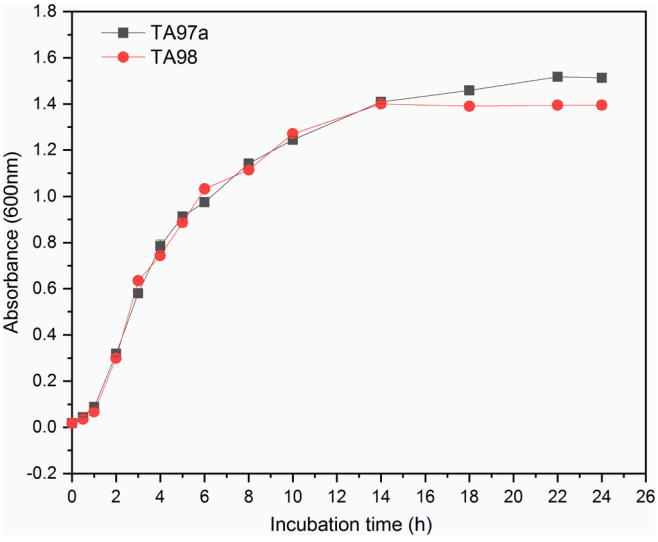

Figure 2. Growth curves of Ames tester strains (TA97a and TA98) based on broth medium.

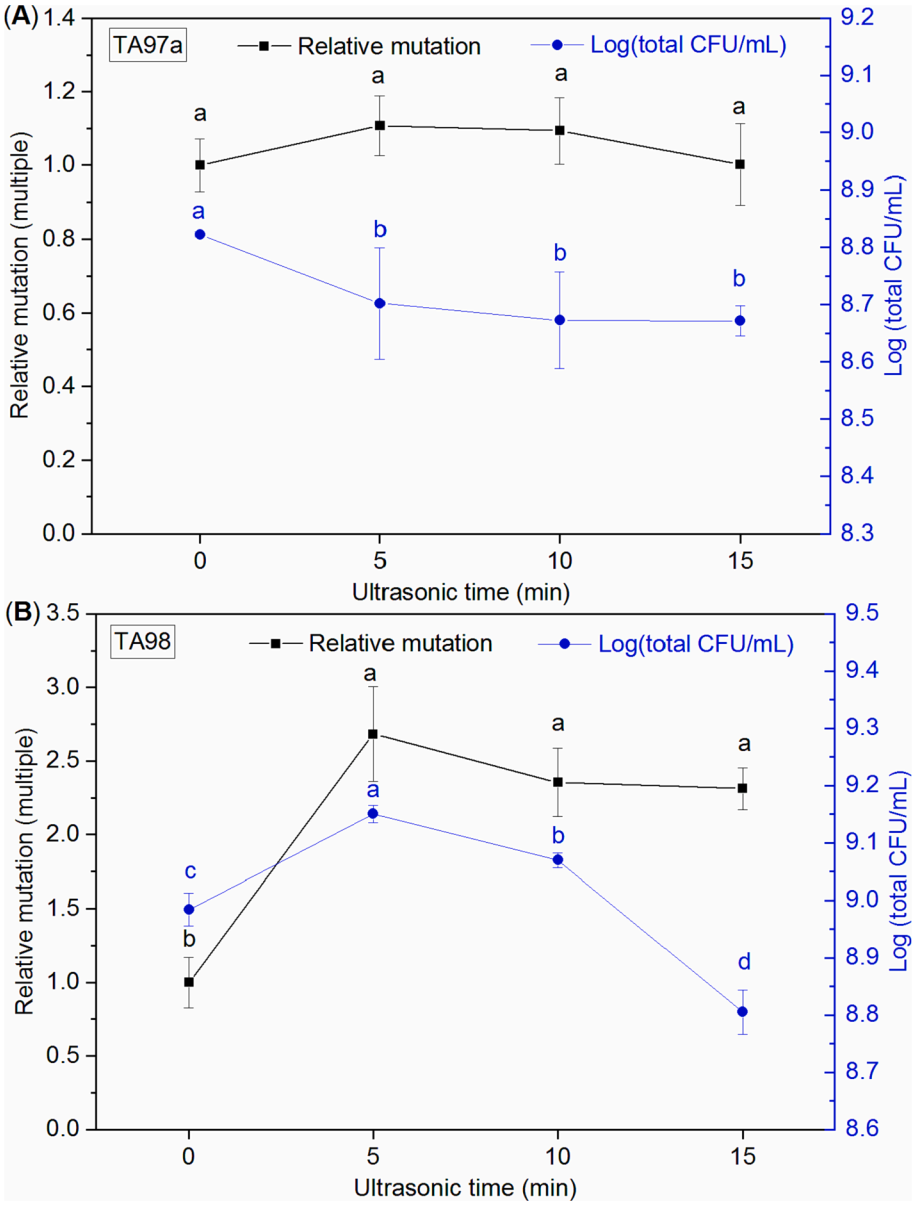

Figure 3. Effects of ultrasonic time (0, 5, 10 and 15 min) on reversion mutation and total CFU of TA97a (A) and TA98 (B) after cultivation for 6 h. The relative mutation was the ratio of mutation rate of ultrasound group and blank group. Sonication conditions: ultrasonic pulsed model of on-time for 4 s and off-time for 1 s under ultrasonic power density of 20 W/L and frequency of 40 kHz.

mutagens [32,33]. This was probably explained by the different InDel types in *hisD3052* gene of TA98 (defective gene of −1 bp deletion of CCC → CC) and TA97a (defective gene of +1 cytosine at run of C's) [20,34]. Therefore, TA98 was selected for further experiment.

Furthermore, in this study, ultrasound did not cause a substantial reduction in the total number of colonies (< 0.3log), which suggested that low-intensity ultrasound was non-lethal in the process of promoting the reversion mutation (InDels) of TA98 strain (some ultrasonic conditions promoted the growth of strains) and it was considered as a weak mutagenesis technology suitable for continuous mutation. This was dissimilar to those traditional strong mutagenic fields, such as UV, ARTP and X-ray, in which the lethal rate is often applied to evaluate the mutagenic effects. However, traditional strong mutagenic fields were prone to excessive mutagenesis of strains and thus affected the comprehensive performance of mutants, which was not conducive to the adaptive survival of the mutants. Adaptive laboratory evolution (ALE) is a continuous evolutionary mutation and an efficient strategy for adapting original strain under the given selective pressure to screen mutants with improved phenotypes or physiological characteristics [17]. Traditional ALE is mainly concentrated on the natural mutation of gene, which needs many circles of passage to obtain expected mutants suitable for environmental factors. If the low-lethality ultrasound technology is continuously applied to evolutionary mutagenesis, it will enhance mutation efficiency and promote the production and enrichment of positive mutation. Accordingly, our findings provided important theoretical references for the application of ultrasound in evolutionary mutation.

3.3. Effects of ultrasound parameters on reversion mutation of TA98 strain

3.3.1. Ultrasonic stage

Effects of sonication stage (power density of 20 W/L and frequency of 40 kHz for 5 min) on the reversion mutation and total CFU/mL of *Salmonella typhimurium* TA98 in the metaphase of logarithmic growth phase (incubation for 4, 6 and 8 h) were plotted in Fig. 4A. The results reflected

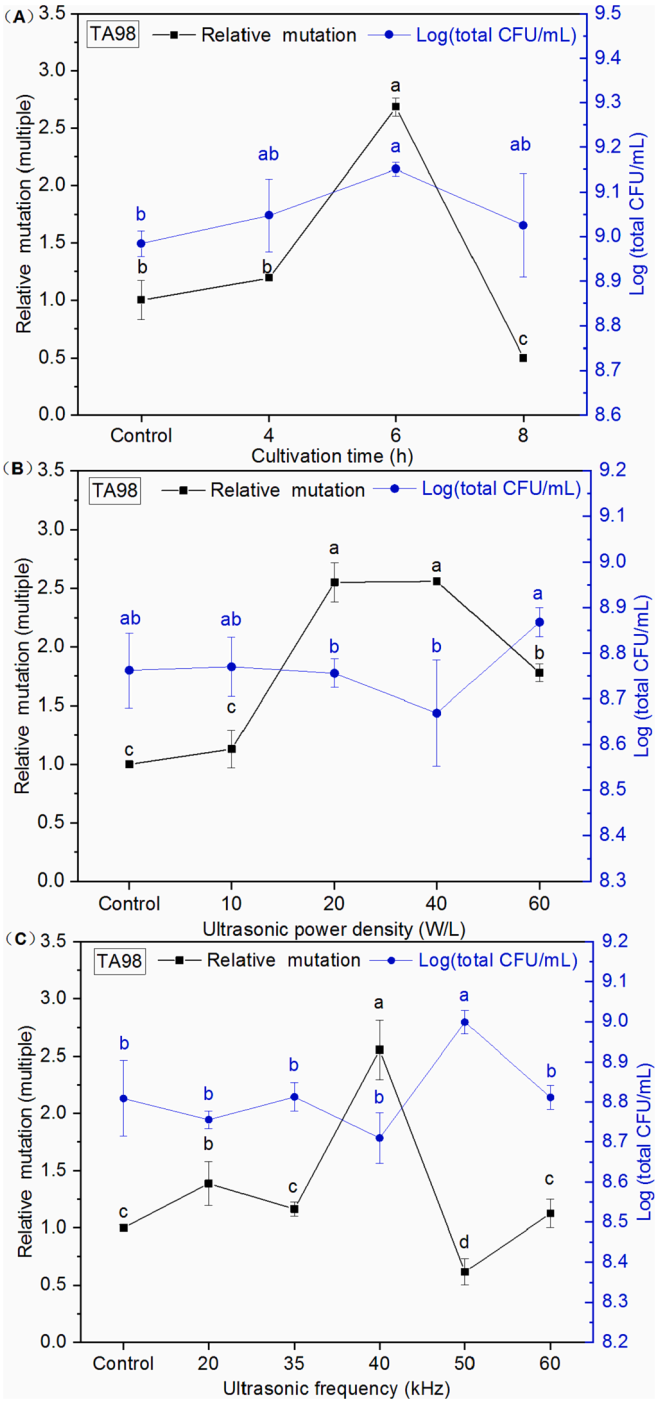

Figure 4. Effects of ultrasonic stage (A), power density (B) and ultrasonic frequency (C) on reversion mutation and total CFU of TA98 for 5 min with ultrasonic pulsed model of on-time for 4 s and off-time for 1 s. The relative mutation was the ratio of mutation rate of ultrasound group and blank group.

that sonication exerted on TA98 strain after cultivation for 6 h significantly increased the reversion mutation rate (up to 1.1×10^{-6}) and total CFU/mL, which enhanced by 168.25% and 45.08% over the control, respectively ($p < 0.05$). In the early stage of cultivation, TA98 strain acclimatized themself to synthesize and accumulate sufficient amounts of enzymes or intermediate metabolites for cell proliferation [18]. Thus, the introduction of ultrasound at cultivation for 4 h was not obvious to stimulate cell growth and reversion mutation. But sonication at later cultivation period of 8 h was also not conducive to the improvement of reversion mutation, which was probably ascribed to the nutrient deprivation or stressing conditions [19]. Compared to the control, regardless of sonication at cultivation for 4 h or 8 h, there was no significant increment in the total CFU/mL of TA98 strain, which was inconsistent with the results of Dai et al. [1] who discovered that the biomass of *Saccharomyces cerevisiae* increased by 127.03% after sonication for 1 h at frequency of 28 kHz and power of 140 W/L. This may be attributed to the difference of ultrasonic conditions and bacterial strains. Therefore, sonication exerted on TA98 strain after cultivation for 6 h was applied in the further experiment.

3.3.2. Ultrasonic power

Fig. 4B displayed the influence of ultrasonic power density (10, 20, 40 and 60 W/L) on the reversion mutation rate and total CFU/mL of TA98 at frequency of 40 kHz for 5 min after cultivation for 6 h. Compared to the control, sonication at power density of 10 W/L did not lead to the measurable increment of TA98 revertants. The maximum multiple (2.55) of relative mutation over the spontaneous reversion rate appeared at sonication of 20 W/L and 40 W/L. With the further increasing of ultrasonic power density to 60 W/L, a decreased relative mutation multiple (1.78) was found in TA98 strain above the control. Hence, higher power density (> 40 W/L) were not always needed to obtain the desired mutation effects. However, the total CFU/mL of TA98 to receive sonication at 60 W/L was more than those of 20 W/L and 40 W/L. This suggested that power density of more than 40 W/L was not conducive to promoting reversion mutation, but it was beneficial for cell growth of TA98 strain. It could be deduced that there were probably different ultrasonic response mechanisms for regulating DNA mutation and cell growth. Additionally, high power of sonication easily causes worsening of the ultrasonic transducer, which led to too much cavitation and poor transmission of the ultrasound through the liquid media [20]. Overall, 20 W/L was selected as the optimal sonication power density to promote reversion mutation of TA98 strain in the further experiments.

3.3.3. Ultrasonic frequency

Effects of ultrasonic frequency (20, 35, 40, 50 and 60 kHz) on reversion mutation and total CFU/mL of TA98 strain at 20 W/L for 5 min after cultivation for 6 h were shown in Fig. 4C. The maximum relative mutation of 2.56 multiple (reversion mutation rate of 1.0×10^{-6}) was exhibited at ultrasonic frequency of 40 kHz, followed by 20 kHz (1.39 multiple). It could be seen that simply increasing the frequency of sonication did not obtain more reversion mutations. This was probably attributed to the so-called “frequency windows”, which could provide special biological effects on cellular system [21]. According to the investigation, there was no significant difference in total CFU/mL for TA98 between sonicated samples and the control except sonication of 50 kHz, where the reversion mutation rate was lower than spontaneous reversion rate. This indicated that ultrasound frequency not conducive to promoting reversion mutation was better for cell growth, which was consistent with the results of ultrasound power density. These results provided important references for rational selection of ultrasound parameters to meet different experimental needs. Different from previous researches that reported ultrasound could promote the propagation of microorganism and thus lead to biomass increment [1,3], in this study, the shorter ultrasonic treatment time was enough to promote the reversion mutation rather than discernible proliferation of *Salmonella typhimurium* TA98. This could be explained by the spontaneous reversion of Ames tester strains and this process could be accelerated by the introduction of ultrasound. Collectively, 40 kHz was regarded as the optimal sonication frequency for promoting reversion mutation of TA98 strain.

3.4. Ultrasound-mediated InDels polymorphisms in hisD3052 gene of TA98 revertants

The InDels in *hisD3052* gene of total 1752 TA98 revertants from sonication group (frequency of 40 kHz, power density of 20 W/L for 5

min after cultivation for 6 h) and the control were studied based on sequencing analysis. As can be seen in Fig. 5A, there were base insertions (+43/+34/+28/+25/+22/+19/+16/+10/+7/+4/+1 bp) and deletions (−2/−5/−8/−11/−14/−17/−20/−23/−29 bp) of InDels observed in *hisD3052* gene of TA98 revertants, which was much similar to lognormal distribution and inconsistent with those reported previously [14]. Additionally, all InDels of sonicated TA98 revertants appeared to be double frameshifts (+/−), which proved to be shown in Fig. 5B. This was in agreement with the results of Isono *et al.* who discovered revertants were double frameshift (+/−) mutants induced by different carcinogens [14]. These InDels were conformed to the general regularity of 3*n*+1 (*n* is an integer). This was attributed to the fact that three base codons corresponded to one amino acid and the (3n+1) bp could restore the reading frame of the gene encoding protein because there was −1 bp deletion in h*isD3052* gene of TA98 strain compared to the wild-typed strain [14]. However, there were neither insertions of +40/+37/+31/+13 bp nor deletion of −26 bp detected in this experiment, which was probably ascribed to the no restoration of protein function caused by these InDels in *hisD3052* gene of TA98 strain [13].

The classical InDel (+1 bp) of *hisD3052* gene in TA98 revertants from the control and sonication group accounted for 17.81% and 12.45%, respectively, of which the percentage of *hisD3052* gene reversion to the wild-typed sequence (CC→CCC) was in the tiny minority for both groups (0.34% and 0.23% for sonication group and the control group, respectively). In nonclassical InDels, +4 bp insertion and −2 ~ −23 bp deletions (almost 80% including −2 bp of 55.60%) occurred in the control group. After sonication, there was an additional deletion of −29 bp (1.60%) and insertions of +7 ~ +43 bp direct repeat sequences (16.10%) observed in *hisD3052* gene of TA98 revertants (Table 2), the proportion of which +16 bp (4.22%) and +28 bp (2.85%) were significantly higher than other adjacent insertion types. This indicated that ultrasound promoted the occurrence of base insertions, especially the insertions of DNA large fragments, which rarely occurs spontaneously. Furthermore, the insertions of +7 ~ +43 bp direct repeat sequences were consistent with the law of micro-homologous recombination [22,23]. This could be explained by the InDels caused by a less direct mechanism than that of intercalating agents [24]. Regardless of the control (without sonication) or sonication group, −2 bp deletion was the main InDel in *hisD3052* gene for TA98 strain. *A priori*, TA98 strain (−1 bp deletion) should be reversion to the wild-typed gene (CC→CCC) in *hisD3052* because the correction of +1 bp insertion may occur by restoration of the wild-typed base sequence. However, this study showed that −2 bp deletion was the predominant InDel type in *hisD3052*, which implied that this capacity of +1 bp insertion was much weaker than its capacity to cause −2 bp deletion in similar DNA repeats for TA98 strain. This indicated that the probability of base deletion of InDels polymorphisms was higher than that of base insertion in the process of DNA replication.

Base on the sequencing results of 1752 TA98 revertants, it was found that there was a total of 127 InDel types (Fig. 6A). The common InDel types from ultrasound group and the control were 57 kinds, and the unique InDel types of ultrasound group were 53 kinds, which was 36 kinds more than that of unique to the control. This illustrated that ultrasound could significantly increase the InDel types. In the sonication group, there was only one mutation type (unique to ultrasound) observed in the +16 ~ +43 bp insertions and −29 bp deletion, while 3

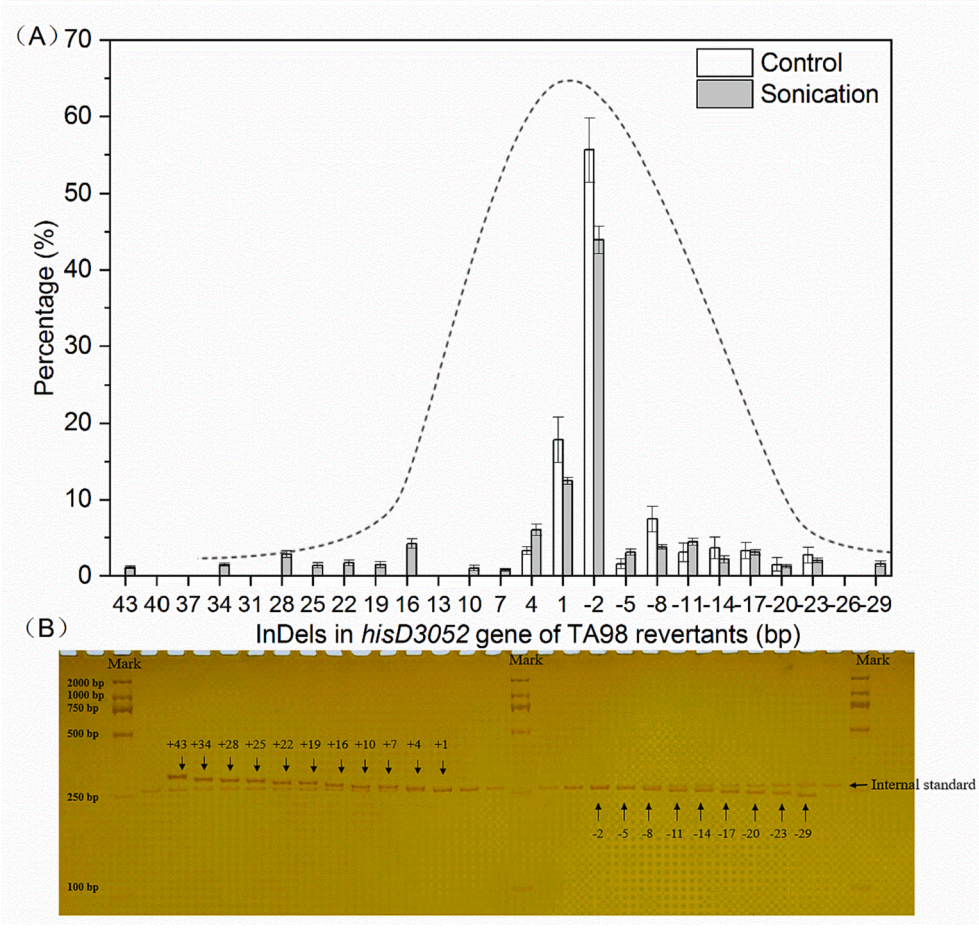

Figure 5. Distribution of InDels polymorphism based on sequencing results of *hisD3052* gene from TA98 revertants (A) and PAGE of the corresponding InDels types of PCR products from *hisD3052* gene (B).

Table 2
DNA and amino acid sequences of core mutation region in *hisD3052* gene from TA98 revertants (unique to ultrasound)

Types	DNA sequence	Amino acid sequence
TA98	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTGGCGGACGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC (No. 832 ~ 915 bp)	ARKVAEAVERQLAELPRADTAGRP(End)
Wild-typed	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTGGCGGACGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAELPRADTARQALSAS
+43 bp	GCCCGCAAGGTGGCGGAGGCGGTAGA(ACGTCAACTGGCGGACGCGGACACCGCCGGCAGG)$_2$GCCCTGAGCGCCAGTC	ARKVAEAVERQLAELPRADTAGRTSTGGTAARGHRRQALSAS
+34 bp	(GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAAC)$_2$TGGCGGAACTGCCGCGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQRPQGGGGGRTSTGGTAARGHRRQALSAS
+28 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAA(CTGGCGGAACTGCCGCGCGGACACCG)$_2$CCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAELPRADTAGGTAARGHRRQALSAS
+25 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTGGCGGACGCGGACAC)$_2$CGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAELPRADTGGTAARGHRRQALSAS
+22 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTGCCGCGCGGACACCGCC)$_2$GGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAELPRADTATAARGHRRQALSAS
+19 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTC(AACTGGCGGAACTGCCGCG)$_2$CGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAELPRADTAARGHRRQALSAS
+16 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTGCGCGGAACTGCCG(CGCGCGGACACCGCCG)$_2$GCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAELPRADTAARGHRRQALSAS
+10 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCA(ACTGGCGGAA)$_2$CTGCCGCGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAETGGTAARGHRRQALSAS
	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAA(CTGGCGGAAC)$_2$TGCCGCGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAEPGGTAARGHRRQALSAS
	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTGCCGCGCGGACACCGCC(GGCAGGCCCT)$_2$GAGCGCCAGTC	ARKVAEAVERQLAELPRADTAGRPWQALSAS
+7 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAA(CGTCAAC)$_2$TGGCGGAACTGCCGCGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQPSTGGTAARGHRRQALSAS
	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTG(GCGGAAC)$_2$TGCCGCGCGGACACCGCCGGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQLAERGTAARGHRRQALSAS
−29 bp	GCCCGCAAGGTGGCGGAGGCGGTAGAACGTCAACTGGCGGAACTGCCGCGCGGACACCGCC)$_6$GGCAGGCCCTGAGCGCCAGTC	ARKVAEAVERQRQALSAS

The number at the bottom right of the brackets represents the number of repetitions of the sequence in the bracket.

and 2 types of mutation (unique to ultrasound) were observed in the +10 bp and +7 bp insertions, respectively. Except for InDel types in common (common to both), the respective unique InDel types from both groups were observed in the +4 bp, +1 bp, −2 bp, −8 bp, −11 bp and −14 bp, while in the −5 bp, −17 bp, −20 bp and −23 bp (>1 kind), the unique InDel type only appeared in the sonication group. This was considered as a validation that the restoration of protein function induced by nonclassical InDels in *hisD3052* gene also caused the phenotypic reversion mutation of TA98 strain. This aspect of the research suggested that ultrasound increased more mutation possibilities, which has been mainly attributed to ultrasonic physical effects (acoustic cavitation and the resulting high-speed microjets and shockwaves) and/or chemical phenomena occurred owing to the formation of free radicals from the sonolysis of water vapor inside collapsing bubbles [25–27].

3.5. Effect of ultrasound on mRNA expression profile of TA98 strain

In order to better understand ultrasound-mediated InDels in *hisD3052* gene of TA98 strain, this study specifically analyzed the differential gene expression in mRNA transcription process of TA98 at cultivation for 0 min and 10 min after ultrasound treatment. In this experiment, four groups of TA98 strain were prepared for Illumina transcriptome sequencing. Each group contained three samples with the same treatment (total 12 samples). Two group was treated with the optimal sonication conditions for cultivation at 37 °C for 0 min and 10 min (T_0min and T_10min), respectively; the other two group was untreated as a control group for cultivation at 37 °C for 0 min and 10 min, respectively (C_0min and C_10min). Through normalization and hierarchical clustering, the clustering heat map of differentially expressed genes was drawn after GO enrichment analysis between sonication group and control group (Fig. 7A). According to the differential genes KEGG enrichment analysis, the enriched KEGG pathways at 0 min (8 up-regulated pathways and 13 down-regulated pathways) and 10 min (35 up-regulated pathways and 7 down-regulated pathways) were respectively displayed in Fig. 7B and C.

As can be seen in Fig. 7A, these differentially expressed genes involve a variety of key genes related to DNA replication and repair process. The involved representative differentially expressed genes mainly contained *topA* (GO: 0018124), *uvrD* (GO: 0000724), *hupA* (GO: 0003677), *polA* (GO: 0015426), *polB* (GO: 0042575), *holB* (GO: 0006261), *lig* (GO: 0015989), *recO* (GO: 0000809), *recN* (GO: 0009432), *radA* (GO: 0000794), *mutL* (GO: 0032390) and *mutS* (GO: 0062128) according to their *p*-value (< 0.05). The downstream proteins transcribed and translated by the genes of *topA*, *uvrD* and *hupA* respectively corresponded to the DNA topoisomerase, DNA helicase and DNA-binding protein (SSB, single-stranded DNA binding protein), which binds to the single-stranded DNA to prevent DNA rematching and protect it from degrading by protein or nucleic acid enzymes [28]. These three proteins jointly regulate the unwinding of double strands in the process of DNA replication, which is vital for cell growth, repair and reproduction in organisms [29]. In this study, the overall expression of DNA helicase, SSB and DNA ligase (lig, GO: 0015989) decreased significantly, indicating that ultrasound may cause the instantaneous stagnation of DNA replication-fork.

The genes of *polA*, *polB* and *holB* belong to the ontology of DNA polymerases, which play pivotal roles in the complex processes that maintain genetic integrity [30]. The mRNA of DNA polymerases was first down-regulated (0 min) to about 2 times and then up-regulated (10 min) in the sonicated TA98 strain to about 2 times as compared with that of the control. The result further suggested that ultrasound changed the expression of genes related to DNA replication. Combined with the up-regulated expression in *recO* (recombination protein) and *recN*, which is a kind of SOS-inducible protein and serves to stabilize damaged DNA prior to recombination and repair [31], ultrasound treatment influenced the process of DNA replication by making DNA replication-fork stop

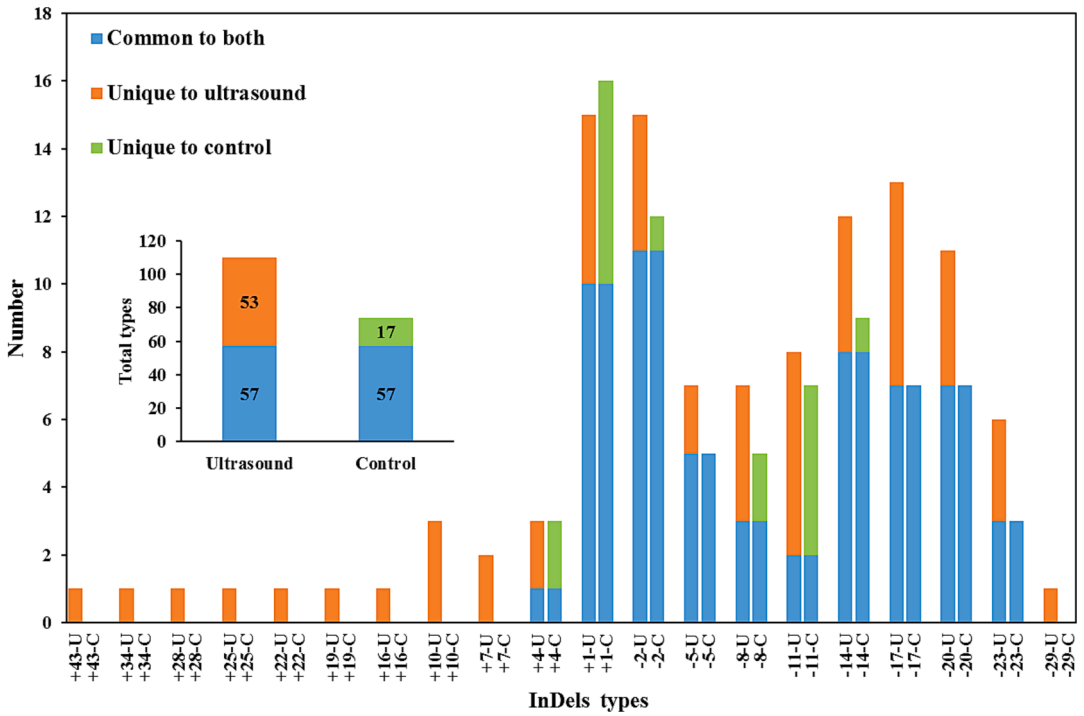

Figure 6. Number of types of each InDel in *hisD3052* gene from 1752 TA98 revertants. U – ultrasound group, C – the control group (without sonication).

instantaneously, and the activation of DNA related repair mechanism ensured the normal progress of DNA replication. Additionally, there was a significant up-regulation expression in DNA mismatch repair genes *mutL* and *mutS*, which could also provide products for transcription-coupled nucleotide-excision repair [32]. Different from the DNA mismatch repair encoded by *mut* genes, the up-regulated repair protein encoded by radA gene is mainly involved in homologous recombination and DNA damage repair [33]. These findings indicated that sonication affected the DNA replication process and then initiated the corresponding DNA repair mechanisms during the reversion mutation of TA98 strain.

According to the KEGG pathway enrichment analysis, the involved pathways of these differentially expressed genes at 0 min (Fig. 7B) were only half of that at 10 min (Fig. 7C), at which the up-regulated pathways increased by 27 pathways compared with those of 0 min. This indicated that it was necessary to select the appropriate mRNA transcription monitoring time after ultrasound treatment, because DNA replication was a continuous dynamic process. The activation of DNA damage repair pathway occurs only after DNA damage, followed by the activation of cell cycle nodes [34]. Based on the *p*-value (< 0.05) and the functions of the ultrasound reflected on the phenotypes of the sonicated TA98 strain, four representative KEGG pathways were observed in the two-component system (path: ko02020) and DNA repair mechanisms including base excision repair (path: ko03410), mismatch repair (path: ko03430) and homologous recombination (path: ko03440).

Two-component system is a predominant signalling mechanism and comprises histidine kinases, histidine phosphotransferases and response regulators, which serves as a basic stimulus–response coupling mechanism that allows organisms to sense and respond to environmental changes [35]. In this study, the up-regulation of overall expression in two-component system suggested sonication probably influenced the frequency of initiation of transcription of specific genes or operons [36] and mediated a cellular response of TA98 strain. The high fidelity of DNA semi-conservative replication is achieved by two cellular functions that involve discrimination of correct *versus* incorrect nucleotides by DNA polymerases [37,38] and postreplication mismatch repair, which is mainly repaired by *mutS*, *mutH* and *mutL* proteins encoded by *mut* gene [39]. In this study, the genes *mutS*, *mutL* and *radA* were up-regulated to about 2 times in the sonicated TA98 strain (Fig. 7), which implied that repair mechanisms probably induced the InDel polymorphisms in *hisD3052* gene of TA98 strain. Furthermore, investigations reported that *RecJ* gene tended to participate in the long-patch base excision repair pathway [40], increasing the possibilities of InDels in *hisD3052* gene of TA98 strain.

3.6. Hypothesis

Combined with the "thermal breathing" effect in DNA replication [41,42] and micro-homologous recombination [22], the mechanism of ultrasound-mediated InDel polymorphisms was put forward (Fig. 8). All the InDels occurred in the core mutation region (No. 832 ~ 915 bp) of *hisD3052* gene (total 1305 bp) and the sequences of InDels unique to ultrasound were shown in Fig. 8A. The inserted DNA large fragments (+7 ~ +43 bp) induced by ultrasound were all direct repeat sequences and there was more than one kind in some insertion types, such as +7 bp and +10 bp (Table 2). This suggested that the ultrasound-mediated base insertions of InDels were not random. From prokaryotes to eukaryotes, the number of repeats sequences in genomes is increasing, which implies that repeats sequences are not junk sequences and their functions have been gradually revealed, including gene expression, transcriptional regulation, chromosome construction and physiological metabolism [43,44]. Therefore, research on the direct repeat sequences induced by low-intensity ultrasound will be not only helpful to further explore the mechanism of ultrasound-mediated InDels, but also conducive to the further development of of life science.

According to the relative structure analysis tools, it was found that the single-stranded DNA from core mutation region (No. 832 ~ 915 bp) of *hisD3052* gene easily formed into a relatively stable hairpin structure (Fig. 8B), which was mainly formed by self-pairing of bases. Based on the prediction analysis, it was observed that an advanced structure formed between the sense DNA of inserted direct repeats sequence in *hisD3052* gene of TA98 revertants and antisense DNA in *hisD3052* gene of TA98 strain (Fig. 8C). The partial bases of inserted direct repeats sequence formed DNA hairpin structure by self-folding, and partial bases

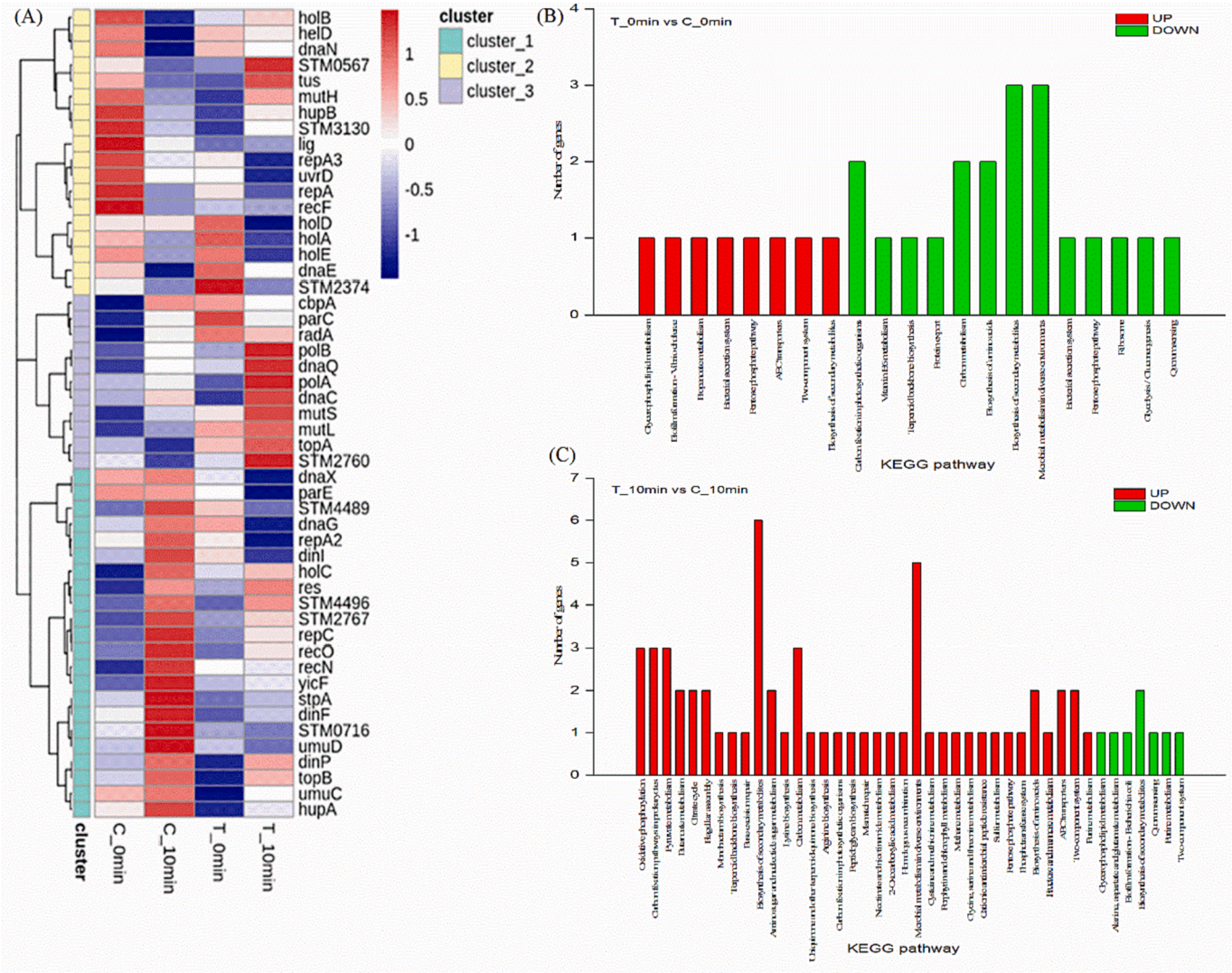

Figure 7. Effects of ultrasound under the optimal sonication conditions on the mRNA expression profile of TA98 strain. (A): Differentially expressed genes heat map; (B): Differential genes KEGG enrichment analysis at cultivation for 0 min after sonication; (C): Differential genes KEGG enrichment analysis at cultivation for 10 min after sonication. (samples of C_0min and C_10min respectively referred to the control groups incubated at 37 °C for 0 min and 10 min; samples of T_0min and T_10min respectively referred to the sonication groups incubated at 37 °C for 0 min and 10 min).

formed micro-homologous pairing with the antisense DNA in *hisD3052* gene of TA98 strain, which was similar to the microhomology-mediated gene recombination [22,23]. Furthermore, combined with the probability of each insertion type in InDels of sonication group (Fig. 5A), the four bases at the 3' end of hairpin structure (Fig. 8C) were observed to regularly conform to a phenomenon. For the same insertions, supposing *m* is the maximum number of base-pairing with the antisense DNA in *hisD3052* gene of TA98 strain. The larger *m* is (the more stable structure with micro-homologous pairing is), the higher is the frequency of occurrence of the corresponding revertants. In this study, the higher proportion of +4, +16 and +28 bp insertions (Fig. 5A) may be related to the existence of more stable micro-homologous sequences. As a whole, the effect of ultrasound on base insertions of InDels was significantly greater than that on base deletions. Therefore, the underlying mechanisms were proposed as follows:

(1) Mechanisms of base deletions in InDels based on ultrasonic mass-transfer effect

In the process of DNA bi-directional replication (5́→3́), SSB functions as a sliding platform that migrates on DNA via reptation [45] and can spontaneously bind to or shed from the single-stranded DNA [46], which is prone to make complementary base-pairs of single-stranded DNA meet by self-folding and form hydrogen bonds, thus resulting in the formation of local hairpin structure (Fig. 8D). Accordingly, DNA polymerase III could cross this region in DNA replication, which resulted in the deletion of this fragment (Fig. 8D). In this study, the average coefficient of variation (CV value of 16.51%) of base deletions induced by ultrasound was much less than that of the control (CV value of 32.79%), which demonstrated that the distribution of base deletions induced by ultrasound was more uniform (Fig. 5A). This was probably due to the high frequency vibration induced by ultrasound that accelerated the balance of SSB shedding from and binding to DNA single strand. It was reported that SSB tetramers could bind to single-stranded DNA in several binding modes, (SSB)x, where the number of nucleotides occluded per tetramer (x) was 34 or 35 [47,48]. In this study, the most deletion of −29 bp illustrated that ultrasound probably caused one SSB to shed from DNA single strand.

(2) Mechanisms of base insertions in InDels based on ultrasonic heat-transfer effect

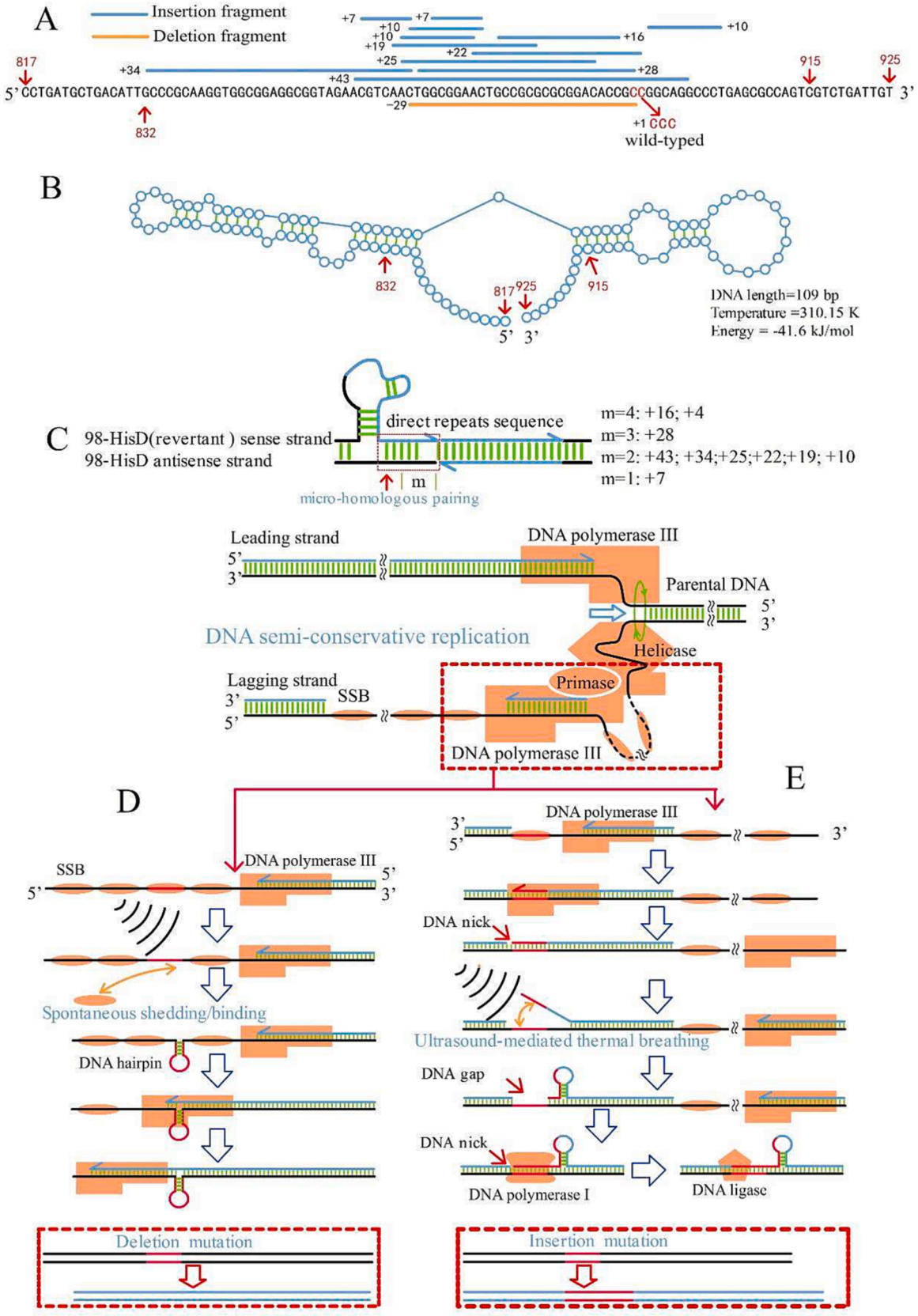

Figure 8. Proposed mechanisms of ultrasound-mediated InDels in *hisD3052* gene. (A): InDel polymorphisms (unique to ultrasound) of core mutation region (No. 832 ~ 915 bp); (B): Structure prediction of single-stranded DNA hairpin of core mutation region; (C): Advanced structure derived from the sense DNA of inserted direct repeats sequence in revertants pairing with antisense DNA of TA98; The proposed base deletion (D) and insertion mechanism (E) of DNA induced by ultrasound.

In the process of DNA bi-directional replication (5́→3́), the leading strand will be directly synthesized after separation of parental DNA strands by the helicase; while the lagging strand is not one-time synthesized as one long strand, but a collection of a large number of small Okazaki fragments. After one Okazaki fragment is synthesized, DNA polymerase III must periodically jump back to the replication fork of the new unwound lagging strand for the next Okazaki fragment. Under the action of instantaneous high temperature generated by ultrasound cavitation, the hydrogen bonds between complementary base-pairs of double-stranded DNA at the nick of Okazaki fragment pair and break periodically ("thermal breathing") [41,42], resulting in the formation of unstable single-stranded DNA (Fig. 8E). When complementary base-pairs of single-stranded DNA meet by self-folding and form hydrogen bonds (hairpin structure), DNA new strand generates a gap which will be filled by DNA polymerase I or other DNA polymerases. Afterwards, the left DNA nick is connected by DNA ligase. Thus, new DNA has additional direct repeats sequence (Fig. 8C) compared to the original sequence.

4. Conclusions

In this study, Ames tester strain was employed as model microorganisms to investigate ultrasound-mediated InDel polymorphisms and underlying mechanisms. Results revealed that sonication promoted the reversion mutation of TA98 strain and the occurrence of InDels, particularly DNA large fragment insertions (+7 ~ +43 bp insertions of direct repeat sequences). Based on the analysis of transcriptomics and prediction of secondary structure of single-stranded DNA from InDels core region (No. 832 ~ 915 bp) in *hisD3052* gene, the mechanisms of ultrasound-mediated InDel polymorphisms of DNA were put forward, which referred to the effects of ultrasonic mass-transfer (SSB shedding from and binding to DNA single strand) and heat-transfer ("thermal breathing"). This finding will shed light on how base sequences prone to forming hairpin structure induce InDels in DNA replication. This may be considered a promising study of the rational design for InDels of DNA large fragment induced by low-intensity ultrasound. We believe that this study will facilitate the in-depth application and development of low-intensity ultrasound technology for continuous mutagenicity.

CRediT authorship contribution statement

Yunliang Li: Software, Methodology. **Siyu Ruan:** Visualization, Conceptualization, Validation, Data curation, Writing – original draft. **Feng Lu:** Writing – review & editing. **Pengfei Xie:** Writing – review & editing. **Xiaoshuang Liu:** Writing – review & editing. **Haile Ma:** Project administration, Resources, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data supporting the results of this study are available within the paper and its Supplementary files. Raw data underlying plots in the figures are available from the corresponding author upon reasonable request.

Acknowledgments

This work was supported by Jiangsu Provincial Key Research and Development Program, CN [Grant No. BE2018368], Research Fund for Young Scholars from Jiangsu Polytechnic College of Agriculture and Forestry [Grant No. 2022kj12] and National Natural Science Foundation of China [Grant No. 31701538].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2022.106270.

References

[1] C. Dai, F. Xiong, R. He, W. Zhang, H. Ma, Effects of low-intensity ultrasound on the growth, cell membrane permeability and ethanol tolerance of *Saccharomyces cerevisiae*, Ultrason. Sonochem. 36 (2017) 191–197.
[2] S. Ruan, J. Luo, Y. Li, Y. Wang, S. Huang, F. Lu, H. Ma, Ultrasound-assisted liquid-state fermentation of soybean meal with *Bacillus subtilis*: Effects on peptides content, ACE inhibitory activity and biomass, Process. Biochem. 91 (2020) 73–82.
[3] G. Huang, Y. Tang, L. Sun, H. Xing, H. Ma, R. He, Ultrasonic irradiation of low intensity with a mode of sweeping frequency enhances the membrane permeability and cell growth rate of *Candida tropicalis*, Ultrason. Sonochem. 37 (2017) 518–528.
[4] S. Zhang, H. Cheng, C. Qiu, X. Chen, Effect of ultrasonic mutation on crude polysaccharide of Hericium erinaceus, Food Ferment. Ind. 46 (2020) 126–130.
[5] Y. Li, X. Wang, S. Ruan, A. Zhou, X.Y. Xiaoshuang, H.M. Liu, Advances in the application of ultrasonics technology in microbial engineering, Sci. Technol. Food Ind. 42 (2021) 343–348.
[6] N. Draptchinskaia, B. Gustavsson P Fau - Andersson, M. Andersson B Fau - Pettersson, T.N. Pettersson M Fau - Willig, I. Willig Tn Fau - Dianzani, S. Dianzani I Fau - Ball, G. Ball S Fau - Tchernia, J. Tchernia G Fau - Klar, H. Klar J Fau - Matsson, D. Matsson H Fau - Tentler, N. Tentler D Fau - Mohandas, B. Mohandas N Fau - Carlsson, N. Carlsson B Fau - Dahl, N. Dahl, The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia, Nat. Genet. 21 (1999) 169–175.
[7] Y. Miki, J. Swensen, D. Shattuck-Eidens, P.A. Futreal, K. Harshman, S. Tavtigian, Q. Liu, C. Cochran, L.M. Bennett, W. Ding, A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1, Science 266 (1994) 66.
[8] H. Luo, H. Cao, Research on the mechanism of ultrasonic breeding (Chinese), Tech. Acoust. 28 (2009) 183–184.
[9] K. Mortelmans, E. Zeiger, The Ames *Salmonella*/microsome mutagenicity assay, Mutat. Res-Fund Mol. M. 455 (2000) 29–60.
[10] D. Williams-Hill, C.P. Spears, S. Prakash, G.A. Olah, T. Shamma, T. Moin, L.Y. Kim, C.K. Hill, Mutagenicity studies of methyl-tert-butylether using the Ames tester strain TA102, Mutat. Res-Gen. Tox. En. 446 (1999) 15–21.
[11] A.B. Barbezan, R. Martins, J.B. Bueno, A.L.C.H. Villavicencio, Ames test to detect mutagenicity of 2-Alkylcyclobutanones: A review, J. Food Sci. 82 (2017) 1518–1522.
[12] H.-Y. Kim, J.-H. Lee, M.J. Kim, S.C. Park, M. Choi, W. Lee, K.B. Ku, B.T. Kim, E. Changkyun Park, H.G. Kim, S.I. Kim, Development of a SARS-CoV-2-specific biosensor for antigen detection using scFv-Fc fusion proteins, Biosens. Bioelectron. 175 (2021) 112868–112875.
[13] S. Baghbeheshti, S. Hadadian, A. Eidi, L. Pishkar, H. Rahimi, Effect of flexible and rigid linkers on biological activity of recombinant tetramer variants of S3 antimicrobial peptide, Int. J. Pept. Res. Ther. 27 (2021).
[14] K. Isono, J. Yourno, Chemical carcinogens as frameshift mutagens: *Salmonella* DNA sequence sensitive to mutagenesis by polycyclic carcinogens, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 1612–1617.
[15] B.N. Ames, M.C. Yamasaki, Methods for detecting carcinogens and mutagens with the *salmonella*/mammalian-microsome mutagenicity test, Mutat. Res. Environ. Mutagen. Relat. Subj. 31 (1975) 347–364.
[16] K. Weber, M. Osborn, The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis, J. Biol. Chem. 244 (1969) 4406–4412.
[17] M. Dragosits, D. Mattanovich, Adaptive laboratory evolution-principles and applications for biotechnology, Microb. Cell Fact. 12 (2013) 64.
[18] A. Martinez-Antonio, J. Lomnitz, S. Motta, M. Aldana, M. Savageau, Regulatory design governing progression of population growth phases in *bacteria*, PLoS One 7 (2012) e30654.
[19] D. Houle, K.A. Hughes, S. Assimacopoulos, B. Charlesworth, The effects of spontaneous mutation on quantitative traits. II. Dominance of mutations with effects on life-history traits, Genet. Res. Camb. 70 (1997) 27–34.
[20] P.B. Subhedar, P.R. Gogate, Intensification of enzymatic hydrolysis of lignocellulose using ultrasound for efficient bioethanol production: A review, Ind. Eng. Chem. Res. 52 (2013) 11816–11828.
[21] M. Zhadin, F. Barnes, Frequency and amplitude windows in the combined action of DC and low frequency AC magnetic fields on ion thermal motion in a macromolecule: Theoretical analysis, Bioelectromagnetics 26 (2005) 323–330.
[22] J.A. Lee, C.M.B. Carvalho, J.R. Lupski, A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders, Cell 131 (2007) 1235–1247.
[23] H. Wang, P. Liu, W. Bi, W. Zhou, S.W. Cheung, Genome rearrangement and human genome diseases (Chinese), Chin. J. Evid. Based Pediatr. 9 (2014) 150–157.
[24] I.J. Yourno, Chemical carcinogens as frameshift mutagens: *Salmonella* DNA sequence sensitive to mutagenesis by polycyclic carcinogens, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 1612–1617.
[25] A. Starek, Z. Kobus, A. Sagan, B. Chudzik, J. Pawłat, M. Kwiatkowski, P. Terebun, D. Andrejko, Influence of ultrasound on selected microorganisms, chemical and structural changes in fresh tomato juice, Sci. Rep. 11 (2021) 3488.
[26] A. Henglein, Chemical effects of continuous and pulsed ultrasound in aqueous solutions, Ultrason. Sonochem. 2 (1995) S115–S121.

[27] S. Wang, R. Feng, X. Mo, The cavitation valley phenomenon of rectangular wave modulation ultrasound, Ultrason. Sonochem. 3 (1996) 69–71.
[28] R.D. Shereda, A.G. Kozlov, T.M. Lohman, M.M. Cox, J.L. Keck, SSB as an organizer/mobilizer of genome maintenance complexes, Crit. Rev. Biochem. Mol. 43 (2008) 289–318.
[29] J.B. Reece, N.A. Campbell, Campbell Biology, 7th ed., Benjamin Cummings, 2011.
[30] U. Hübscher, S. Spadari, G. Villani, G. Maga, DNA Polymerases, World Scientific, 2010.
[31] I. Odsbu, K. Skarstad, DNA compaction in the early part of the SOS response is dependent on RecN and RecA, Microbiology 160 (2014) 872–882.
[32] I. Mellon, G.N. Champe, Products of DNA mismatch repair genes mutS and mutL are required for transcription-coupled nucleotide-excision repair of the lactose operon in *Escherichia coli*, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 1292–1297.
[33] D.L. Cooper, D.C. Boyle, S.T. Lovett, Genetic analysis of *Escherichia coli* RadA: functional motifs and genetic interactions, Mol. Microbiol. 95 (2015) 769–779.
[34] J. Wu, X. Zhang, L. Zhang, C. Wu, A.H. Rezaeian, C. Chan, J. Li, J. Wang, Y. Gao, F. Han, Y.S. Jeong, X. Yuan, K.K. Khanna, J. Jin, Y. Zeng, H. Lin, Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1, Mol. Cell 46 (2012) 351–361.
[35] E.G. Biondi, Two-Component Systems, in: W. Dubitzky, O. Wolkenhauer, K.-.-H. Cho, H. Yokota (Eds.), Encyclopedia of Systems Biology, Springer, New York, New York, NY, 2013, pp. 2305–2306.
[36] M.R. Graham, L.M. Smoot, C.A.L. Migliaccio, K. Virtaneva, D.E. Sturdevant, S. F. Porcella, M.J. Federle, G.J. Adams, J.R. Scott, J.M. Musser, Virulence control in group A *Streptococcus* by a two-component gene regulatory system: Global expression profiling and *in vivo* infection modeling, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 13855–13860.
[37] H. Echols, M. Goodman, Fidelity mechanisms in DNA replication, Annu. Rev. Biochem. 60 (1991) 477–511.
[38] K.A. Johnson, Conformational coupling in DNA polymerase fidelity, Annu. Rev. Biochem. 62 (1993) 685–713.
[39] P. Modrich, R. Lahue, Mismatch repair in replication fidelity, genetic recombination, and cancer biology, Annu. Rev. Biochem. 65 (1996) 101–133.
[40] K. Cheng, Y. Xu, X. Chen, H. Lu, Y. He, L. Wang, Y. Hua, Participation of RecJ in the base excision repair pathway of *Deinococcus radiodurans*, Nucl. Acids Res. 48 (2020) 9859–9871.
[41] B.F. Putnam, L.L. Van Zandt, E.W. Prohofsky, W.N. Mei, Resonant and localized breathing modes in terminal regions of the DNA double helix, Biophys. J. 35 (1981) 271–287.
[42] R. Roychoudhury, C.-P.-D. Tu, R. Wu, Influence of nucleotide sequence adjacent to duplex DNA termini on 3′ terminal labeling by terminal transferase, Nucl. Acids Res. 6 (1979) 1323–1333.
[43] J.A. Shapiro, R.v. Sternberg, Why repetitive DNA is essential to genome function, Biol. Rev. Camb. Philos. Soc. 80 (2005) 227–250.
[44] J.Y. Lu, W. Shao, L. Chang, Y. Yin, T. Li, H. Zhang, Y. Hong, M. Percharde, L. Guo, Z. Wu, L. Liu, W. Liu, P. Yan, M. Ramalho-Santos, Y. Sun, X. Shen, Genomic repeats categorize genes with distinct functions for orchestrated regulation, Cell Rep. 30 (2020) 3296–3311.e3295.
[45] R. Zhou, A.G. Kozlov, R. Roy, J. Zhang, S. Korolev, T.M. Lohman, T. Ha, SSB functions as a sliding platform that migrates on DNA via reptation, Cell 146 (2011) 222–232.
[46] Y. Lu, Studying the binding properties of *E-coli* single-stranded DNA binding protein by single-molecule magnetic tweezers (Chinese), University of Chinese Academy of Sciences, Beijing, 2018.
[47] M.E. Ferrari, W. Bujalowski, T.M. Lohman, Co-operative binding of *Escherichia coli* SSB tetramers to single-stranded DNA in the (SSB)35 binding mode, J. Mol. Biol. 236 (1994) 106–123.
[48] J.B. Clendenning, J. Michael Schurr, A model for the binding of *E. coli* single-strand binding protein to supercoiled DNA, Biophys. Chem. 52 (1994) 227–249.