MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

Bachelor's thesis

on the topic Analysis of the Effect of Histone H3K36Q Point Mutation on Acetic Acid Resistance of Yeast

Completed: student of group BEBT-19
of specialty 162 «Biotechnology and Bioengineering»
Jin LIU 刘进
(Student's First and LAST NAME)

Scientific supervisor Olga Shydlovska
(first name, LAST NAME)

Reviewer Ihor Hrebtskyi
(first name, LAST NAME)

Kyiv 2023

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty Chemical and Biopharmaceutical Technologies
Department Biotechnology, Leather and Fur
Speciality 162 Biotechnology and Bioengineering
Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology,
Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA

«30» 05 2023

ASSIGNMENTS
FOR THE BACHELOR'S THESIS

Jin LIU
(Student's Full Name)

1. Thesis topic Analysis of the Effect of Histone H3K36Q Point Mutation on Acetic Acid Resistance of Yeast

scientific supervisor Olga Shydlovska, PhD
(first name, last name, academic degree, academic title)

approved by the order of the higher educational institution on _____

2. Deadline for student submission of work 15.05.23

3. Initial data for work: Scientific study about acid tolerance of S.cerevisiae, experimental data about H3K36 mutation on acid tolerance in yeast

4. Content of the thesis (list of questions to be developed): introduction, experimental materials, experimental methods, results and analysis, conclusion

5. Date of issuance of the assignments 01.03.23

EXECUTION SCHEDULE

№	The name of the stages of the bachelor's thesis	Terms of performance of stage	Note on performance
1	Introduction	03.05.23	刘进
2	Section 1	05.05.23	刘进
3	Section 2	06.05.23	刘进
4	Section 3	07.05.23	刘进
5	Conclusions	08.05.23	刘进
6	Draw up a bachelor's thesis (final version)	14.05.23	刘进
7	Submission of bachelor's thesis to the department for review (14 days before the defense)	15.05.23	刘进
8	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	28.05.2023	2% – 21% 刘进
9	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	30.05.23	刘进

Student 刘进 (signature) Jin LIU (first name, LAST NAME)

Scientific supervisor (signature) Olga Shydlovska (first name, LAST NAME)

Reviewer (signature) Ihor Hretskyi (first name, LAST NAME)

ABSTRACT

In the rapid development of material economy in modern life, our demand for new energy is also increasing. As the preferred raw material for the preparation of fuel ethanol, lignocellulose provides a good idea to solve the current social problems such as resource exhaustion, environmental pollution and greenhouse effect. *Saccharomyces cerevisiae* has the characteristics of high sugar, high ethanol tolerance, clear genetic background and high ethanol yield, making it the first choice in lignocellulosic ethanol fermentation strain. The complex chemical structure of lignocellulose prevents it from being directly used by microorganisms. Instead, it needs to be pretreated and hydrolyzed to produce monosaccharides before it can be used in fermentation production.

However, after pretreatment and hydrolysis of lignocellulose, in addition to fermentable monosaccharides, a variety of small molecular compounds can be produced to inhibit the growth and metabolism of microorganisms. Acetic acid is one of the most typical small molecule inhibitors, which is highly contained in lignocellulosic hydrolysate. It can directly cross the cell membrane through passive transport, affect the physiological and biochemical reactions of cells, and inhibit cell growth. Histone modification mainly occurs in lysine and serine residues of histones. Common histone modification types include methylation, acetylation, ubiquitination, and cassinization, etc., which can directly or indirectly affect physiological and biochemical processes such as gene expression, DNA replication, DNA repair, etc. In this experiment, we first constructed a mutant strain of histone H3K36, mutated

lysine (H3K36) at the 36th position of histone H3 into glutamine, and glutamine residues could simulate the acetylation of histones, so as to detect the influence of H3K36 simulated acetylation on acetic acid tolerance of *Saccharomyces cerevisiae*. The final results showed that the growth of H3K36Q strain was consistent with that of the wild strain without significant differences, and its acetic acid resistance was not significantly improved compared with that of the wild strain. These results indicated that H3K36 simulated acetylation did not affect acetic acid tolerance of *Saccharomyces cerevisiae*.

The object of the work is H3K36-mutant *Saccharomyces cerevisiae*.

The subject of the work is influence of H3K36 simulated acetylation on acetic acid tolerance of *Saccharomyces cerevisiae*.

The aim of the work is to study influence of H3K36 simulated acetylation on acetic acid tolerance of *Saccharomyces cerevisiae*.

The tasks of the work are to construct a mutant strain of histone H3K36 and to detect the influence of H3K36 simulated acetylation on acetic acid tolerance of *Saccharomyces cerevisiae*.

Key words: Saccharomyces cerevisiae, point mutation, fuel ethanol, acid resistance, H3K36

CONTENT

INTRODUCTION

In modern life, where material development is rapid, our need for energy is increasing. Brewer's yeast has a very important role in the production of ethanol fermentation but is often affected by various factors during the fermentation process, acetic acid produced during the hydrolysis of lignocellulose being one of these factors that can have a negative impact on yeast fermentation. There are also issues that need to be addressed regarding the inability of yeast to use xylose directly and the resulting depletion of resources. This study focuses on further research to analyse yeast resistance to acetic acid by point mutating histone sequences and thereby obtaining yeast acetic acid resistance, the use of resources and the reduction of other wastage due to contamination from phage [1]. It is of great practical importance as well as strong scientific value.

Content: A brewer's yeast histone H3K36Q point mutant strain was constructed, cultured and then studied for the effect of the point mutation on yeast acetic acid tolerance.

Technical route: Mutation is the basis of biological evolution, and with the rapid developments achieved by human research on genes, for mutations can be viewed as two types, spontaneous and induced mutations[2]. A gene in a histone of brewer's yeast is induced to produce a mutation without deletion or insertion, and a successful point mutation is made to phenotypically ferment and study yeast acetate tolerance after the mutation.

At the end of the experiment, the strain with the point mutation grew significantly better than the control under the same conditions by shaking the flask with acetic acid, and there was no major change in the fermentation week, indicating that brewer's yeast acetic acid resistance was not related to the point position.

CHAPTER I
INTRODUCTION

1.1 The industrial value of fuel ethanol

1.1.1 The need for ethanol to replace fossil fuels

Fossil fuels, also called ore fuels, are non-renewable mixtures of hydrocarbons or derivatives of hydrocarbons that are combined together. Fossil fuels produce energy in the process of combustion, thus providing power for production. However, fossil fuels produce carbon dioxide during combustion, which contributes to the greenhouse effect and global warming. In addition, the non-renewable nature of fossil fuels puts mankind at risk of running out of resources. Therefore, the search for a renewable, environmentally friendly, efficient, safe and economical energy source has become a major goal for countries around the world[3].

Fuel ethanol, as an alternative to fossil fuels, has gradually developed into a new green industry with a global annual production capacity of over 80 million tonnes[4]. Ethanol is a safe energy source, and its renewable and low-emission advantages have been widely studied at home and abroad[5]. A major problem in bioethanol production is the availability of raw materials for production[6].

At present, China's fuel ethanol is developing at a very fast pace and has become the world's third largest producer and user of ethanol after the United States and Brazil, and with the multiple contributions of the fuel ethanol industry in the economy, society and the environment, the better use of ethanol has become a very

important research direction. Fuel ethanol is based on lignocellulose as a fuel, it can use agricultural household waste, solve some problems regarding environmental pollution and at the same time reduce greenhouse gas emissions, the advantages of all aspects indicate that fuel ethanol is the optimal alternative to oil[7]. Guiding the economy towards green and low-carbon development[8].

1.1.2 Advantages of fuel ethanol production from lignocellulose

It was only after the outbreak of the first world oil crisis that attention began to be paid to the production of bioethanol[9].Today, the technology for ethanol production from lignocellulose is becoming increasingly sophisticated, Therefore, more and more research is focused on the degradation of wooden cellulose by microorganisms, and researchers have constructed microorganisms with high degradation capacity of wooden cellulose[10]. Pre-treated[11] brewer's yeast can be manipulated by physical and chemical methods to disrupt the tight arrangement of lignocellulose to a certain extent, increasing the effective contact area between the enzyme and the substrate and thus improving the catalytic efficiency.

As the most well studied and widely used microorganism, the tolerance of brewer's yeast as a chassis cell to inhibitors during lignocellulose hydrolysis has become a hot topic. Fuel ethanol is now recognized as the most promising renewable energy source, and brewer's yeast has a high ethanol tolerance and ethanol fermentation capacity in fermentation for ethanol production [12]. Decarboxylation produces acetaldehyde and carbon dioxide, and finally acetaldehyde is used by the

enzyme ethanol dehydrogenase to produce ethanol, a process known as the Crabtree effect [13]. Fuel ethanol can relieve the pressure of energy shortages and is environmentally efficient, with low emissions, and can be used to improve people's quality of life, providing food, clothing and shelter. Expanding the production and use of fuel ethanol is becoming an inevitable trend[14]. It is a low carbon alcohol with excellent combustion characteristics and has the advantage of being highly oxygenated, widely available and renewable, offering a wide range of applications[15].

1.2 Brewer's yeast in lignocellulosic ethanol production

1.2.1 Current status of brewer's yeast

Brewer's yeast is an indispensable microorganism in human's daily life, and has great use value in food fermentation and drinking beverages. Brewer's yeast is already in use for industrial production[16].The global use of brewer's yeast fermentation products of ethanol reached 99 billion L, China's production of about 9.2 billion L, the world's third, after the United States and Brazil [17]. Brewer's yeast is an important microorganism for fuel ethanol production, people have a profound history of research on brewer's yeast, and the utilization of brewer's yeast is very efficient. The production of ethanol from lignocellulosic fermentation has received much academic attention and the production of fuel ethanol can take a great deal of pressure off the oil supply. The development of fuel ethanol is limited by the presence of high levels of acid inhibitors in the lignocellulosic hydrolysate. This has led to further exploration and research.

1.2.2 Physiological properties of brewer's yeast

Brewer's yeast is a eukaryotic micro-organism that grows mainly in acidic environments and is ovoid or spherical in shape, with a double-layered cell wall. Brewer's yeast is a microorganism that is often used in the fermentation process and has a very high tolerance and fermentation capacity, making it a beneficial microorganism that is closely related to humans. Brewer's yeast is rich in nutrients and contains a range of proteins, vitamins, dietary fibres and fats[18]. Brewer's yeast has a short metabolic cycle, is safe, reproduces quickly[19] and is easy to produce on a large scale[20]. Brewer's yeast has been the focus of much research and is used in brewing, feed processing, food and drink[21], as well as in medicine and pharmaceuticals[22]. As a microorganism that has been utilised by humans since early times, the culture conditions of brewer's yeast have been extensively studied, including culture time, inoculum size, temperature and speed, with temperature being an important factor in brewer's yeast fermentation[23]. Brewer's yeast can readily produce ethanol from the fermentation of glucose in a process that involves two major biological processes: the enzymatic involvement of glucose fermentation to pyruvate and the degradation of pyruvate to ethanol. However, brewer's yeast lacks access to xylose and can only use xylose isomers rather than xylose directly, and it is clearly affected by acid inhibitors, so improving the acetic acid tolerance of brewer's yeast has become a key research area.

1.2.3 Advantages of brewer's yeast for lignocellulosic ethanol production

In recent years, the production of fuel ethanol from lignocellulose has become a research hotspot in the development[24] and utilization of biomass resources[25]. With increasingly sophisticated biotechnology, point mutation techniques are also becoming more sophisticated. Brewer's yeast was the first eukaryotic cell to be fully sequenced[26]. Brewer's yeast has been most completely studied in molecular biology and cell biology. At the same time, *Saccharomyces cerevisiae* has a good ethanol tolerance to new products, which also makes it an excellent choice[27]. Point mutation of *Saccharomyces cerevisiae* spots to observe changes in their traits is a convenient and safe method, and can greatly improve the utilisation of *Saccharomyces cerevisiae*, making it less susceptible to external factors when cultured, shortening the fermentation cycle and providing more environmentally friendly energy by producing more fuel ethanol from fermentation.

1.3 The importance of improving the resistance of brewer's yeast to acetic acid

1.3.1 Pretreatment of lignocellulose

In the industrial production of ethanol from lignocellulose, pretreatment is a very important step. Due to the very tight internal arrangement of the lignocellulose, the efficiency of enzyme catalysis is less than 20% and the process is very slow without pretreatment. Therefore, pretreatment is essential in the brewer's yeast fermentation process. In pretreatment, the tightly arranged structure of the

lignocellulose can be disrupted by chemical reagents or pressure temperature[28], resulting in a significant reduction in the degree of polymerisation's and crystallinity and thus a larger contact area between enzyme and substrate, increasing the enzymatic efficiency [29]. The main acid and alkali treatments are used in the chemical pretreatment. Acid treatment is generally pretreated with dilute acid, taking into account instrument corrosion and acid recovery. The average degree of polymerisation of lignocellulose decreases with acid treatment and the reactivity is strong, and acid treatment is the most commonly used pretreatment method. There is also steam blast treatment, where the material is placed in steam that is at high temperature and pressure, and then the pressure is reduced to atmospheric pressure for a very short period of time, thus achieving a pretreatment effect. Lignocellulosic pretreatment needs to be selected depending on the material. Today there are multiple research methods for lignocellulosic pretreatment, but each method has its own advantages and disadvantages, so it is necessary to choose the method that suits the intended material.

1.3.2 Effect of acidic inhibitors on brewer's yeast

Although the cell growth of brewer's yeast is inhibited in an acidic environment[30], when fewer sugars are available, brewer's yeast metabolises acetic acid to acetyl coenzyme A in the medium, thus providing more energy and metabolites through the tricarboxylic acid cycle[31]. And these acids can inhibit the growth of weedy bacteria to some extent[32]. However, at too low a pH, acetic acid

inhibits the metabolism of brewer's yeast, resulting in a longer fermentation cycle. Brewer's yeast plays an important role in the production of ethanol fermentation, but is often affected by various factors during fermentation[33], one of which is the production of acetic acid during the hydrolysis of lignocellulose, which can adversely affect yeast fermentation. Acid inhibitors are usually divided into inorganic and organic acids, and acid inhibitors are also divided into strong and weak acidity in aqueous solutions[34], and the acid resistance of yeast is divided into several categories. Both formic acid and acetic acid are weak electrolytes and both affect the fermentation process of brewing yeast.

1.3.3 Means of improving acetic acid tolerance in brewer's yeast

Acetic acid is a simple monocarboxylic acid and an organic weak acid that has relatively strong antibacterial properties and is often used as a preservative to inhibit the growth of miscellaneous bacteria in various fields such as the food industry. During the fermentation of acetic acid by brewer's yeast, high concentrations of acetic acid have a strong inhibitory effect on the production of ethanol[35]. Under certain conditions, acetic acid can also lead to programmed death or ageing of brewer's yeast[36]. Current research still does not have a characterisation of the development of acetic acid resistance in brewer's yeast, but some studies have shown that brewer's yeast may be able to enhance acetic acid tolerance through single gene overexpression or knockout techniques. In addition, the mechanism of acetic acid toxicity refers to the accumulation of acetic acid due to the breakdown of

acetic acid by lignocellulose, followed by intracellular diffusion, and the inhibition caused by acetic acid differs from that caused by simply lowering pH with acid[37], with the inhibition caused by organic weak acids such as acetic acid having a greater impact. It has been shown that acetic acid inhibits the use of sugars by brewer's yeast[38], thereby inhibiting brewer's yeast fermentation. All of these studies suggest that there is still much room for development of yeast acetic acid tolerance studies.

1.4 Overview of research on histone modifications

1.4.1 Concepts of epigenetics and histone modifications

Epigenetics is a rapidly developing discipline in the life sciences in recent years. Epigenetics is the phenomenon whereby gene expression and expression patterns occur in an organism that can be passed on to offspring while the gene sequence remains unchanged. It is primarily the way in which gene expression and cell survival patterns are affected by modulating the external environment of genes, and these effects may result in certain genes showing different expression patterns in different cells or tissues, resulting in different cell types and characteristics. Such alterations are largely based on the interaction between external environmental changes and internal genetic information and can be passed on to offspring through mitosis or meiosis. Epigenetics and traditional genetics are closely related and interact with each other to form the genetic traits of the offspring. This type of research is involved in microbial and plant and animal growth and development, crop risk resistance levels, disease defence and more. Histone octamers are

composed of four classes of two molecules of each histone proteins[39]. Histones are the main structural proteins of chromosomes and can be classified according to the nature of gel electrophoresis into H1, H2A, H2B, H3 and H4. The H3 and H4 proteins are evolutionarily extremely conserved[40], for example, the H3 of bovine thymus differs by only four amino acids compared to the H3 amino acid sequence of pea[41], and play a crucial role in the structure of nucleosomes. Current studies on histone H3 have focused on post-translational modifications such as methylation, acetylation, phosphorylation and glycosylation[42], which are closely related to DNA replication and DNA repair[43], and such modifications occupy a very important position in genetics. These modifications interact with each other to form a complex system called the histone code[44]. In addition, the sequence-induced mutations in brewer's yeast, which cause changes in the intensity of tolerance to acetic acid, provide some value for subsequent studies of various types of brewer's yeast.

1.4.2 Effect of histone modification on the physiological metabolism of brewer's yeast

Epigenetics is the branch of genetics that can be genetically altered by genes. Histones play a very large role post-translationally and more than 60 amino acid residues have been detected to undergo modifications[45]. The modifications mainly include methylation, acetylation, ADP glycosylation, phosphorylation, and ubiquitination[46]. Under the action of a range of enzymes, these modification sites can interact with corresponding biochemical moieties and are thus covalently modified[47].Such a variety of modifications can exhibit a greater variety of regulatory

information, thus creating a greater number of histone codes. Multiple modifications can also be carried out simultaneously, resulting in the expression of a variety of traits. University professor V. Allfrey first isolated histones by chromatographic separation and then determined the degree of histone acetylation modification by radioactivity assay, and finally found extensive acetylation of histones H3 and H4[48]. Histone H3 is the most modified histone, and this modification plays an important role in balancing transcriptional output[49].The correlations between the modifying effects of histones in yeast are not only linear but also non-linear[50], and the correlations between groups of modifications are stronger. The post-translational regulation of histone modifications at the transcriptional level plays a crucial role, and it is also important to use the study of histone modifications in brewer's yeast to better identify the role of growth in the environment.

1.5 Purpose of the experiment

This article focuses on the construction of a histone H3K36Q point mutant strain using strain PSPZ001 as a vector with transfer fragment and plasmid, and a series of experimental operations including PCR amplification, yeast transformation, Ura dropout, DNA purification and phenotypic fermentation using template S288C as a template, the determination of its growth curve, the analysis of the growth cycle of the point mutant brewer's yeast compared to the control, and thus the analysis of the degree of acetic acid resistance in brewer's yeast.

Summary of the chapter I

1. Lignocellulosic production of ethanol is very important,.Ethanol can be used as a green energy source.

2. Brewer's yeast is very well established in culture and propagation techniques and is very advantageous in the production of ethanol from lignocellulose.

3. The hydrolysis of lignocellulose produces acids which are inhibited by brewer's yeast and it is important to develop acid resistance in brewer's yeast.

4. Point mutations in histones can make a difference in brewer's yeast acetate resistance

CHAPTER II
EXPERIMENTAL MATERIALS

2.1 Experimental apparatus

EP tubes, ultra clean table, pipette gun, ventilated kitchen, glass beads, vortex mixer, centrifuge, centrifuge tubes, PCR instrument, PCR tubes, agarose gel electrophoresis instrument, gel imager, electrophoresis gel observer, microwave oven, constant temperature water bath, autoclave, pH meter, temperature-controlled shaker, constant temperature incubator, spectrophotometer.

2.2 Plasmids and strains

Strain: BSPZ001

Stencil: S288C

2.3 Primers`

The list of primers is shown in Table 1.

Table 1

List of primers used in study

引物名	引物序列
P1	GGAGCCATTTGTTAATATACCG
Ura	CTTGACTGATTTTTCCATGG
Ura-2	CCCTTCCCTTTGCAAATAG

Continuing of table 1

P3	CTTGGTACTAATTCCGGAAG
P4	GTGGTGGATTTTGGAAGG
P6	CTGGAGTAATTTTGAGATTGCGC
P8	GGGGAGATATACCGTAGCAG

2.4 Medium configuration

(1) 5-Fluoroorotic acid medium: Add 0.4g of 5-Fluoroorotic acid and 200ml of distilled water at 50°C to a wide mouth flask. Conduct a water bath to heat it up to melt it.

(2) LB liquid medium: 1% peptone, 0.5% yeast powder, 1% sodium chloride.

(3) YEPD liquid medium: 2% peptone, 1% yeast powder, 2% glucose plus 2% agar (solid medium).

(4) Yeast complete synthesis medium (SC): Yeast basic short source (YN8) 1.7g/L, ammonium sulphate 5g/L, CSM-URA 0.77g/supplement plus uracil to a final concentration of 20mg/L.

(5) Complete synthesis medium for uracil deficiency (SC-URA): yeast basic nitrogen source (YNB) 1.7g/L, ammonium sulphate 5g/L, CSM-URA 0.77g/L, plus 2% agar.

After configuration of the medium adjust the pH, using a pH meter. YEPD medium was adjusted with HCl, others with NaOH, pH adjusted to 6.0-6.5.

Sterilised at 115°C for 30min.

Summary of the Chapter II

1. The use of experimental equipment in the course of experiments is very rigorous.

2. Materials, strains and media must be selected accurately

CHAPTER III
EXPERIMENTAL METHODS

3.1 Extracting the genome of yeast

1) Take ten EP tubes and add 200 μl of DNA extraction solution to each.

2) After sterilisation, transfer to an ultra-clean table and transfer the ten bacteria into the EP tubes with the tip of a pipette, mixing the colonies with the DNA extraction solution.

3) Add 0.4g of glass beads and place them in a ventilated kitchen for 5min.

4) Take the DNA extract 2 and add 200μL of the lower clear solution to each of the EP tubes, taking care to protect it from light.

5) Place in a vortex mixer, level off and then start the work, and remove after 90s.

6) At the end of the process, the centrifuge is replaced, the leveling is started and centrifuged at 1300r for 10min.

7) At the end of centrifugation there is a white shell of dead cells in the middle layer, aspirate the supernatant with a pipette and transfer to a new EP tube with 1 ml of anhydrous ethanol, turn upside down and leave to stand.

8) The tubes were loaded into a centrifuge and leveled and centrifuged at 1300 r for 10 min. After centrifugation, the liquid was poured into a waste jar and dried, followed by the addition of 35 μL of dd water and mixed and stored.

3.2 Yeast PCR amplification

1) Prepare 4 EP tubes and place a single colony in each one, add 50μL NaOH and mix well with the single colony.

2) Fix the EP tubes in the float and place them in a boiling water bath for 10 min.

3) After removal from the water bath, leave to stand until room temperature and centrifuge at 6000r for 1min in a leveling machine, at the end of which the supernatant is aspirated.

4) After removing the supernatant, the enzyme 1 μL KOD, 5 μLBuffer, 1.5 μL primer, 31 μL ddH2O, 2 μL dNTP, 2.5 μL LGSO4, 2.5 μL DMSO were added simultaneously to the PCR instrument for the on-board reaction.

3.3 Agarose gel electrophoresis

1) Firstly, prepare the gel in a triangular flask by weighing 0.2 g of agarose and adding 25 ml of 1 x TAEBuf buffer.

2) Then melt in a microwave oven, bring to a boil and repeat three times, shake well until the gel is clear and free of impurities.

3) Shake well and add 2.5 μL of nucleic acid dye and mix well.

4) The comb is fitted correctly and the glue is poured evenly into the glue plate and left to set for 20min.

5) When the gel is completely solidified, transfer the gel bath with the gel in it to the electrophoresis bath and add 1×TAEBuf buffer to submerge the gel plate.

6) Mix 2μL of 10×DANloading Buffer with the DNA sample to be tested,

and then use a pipette gun to evenly punch it into the sample wells along the edge of the gel wells, trying not to break the loop of gel and being fast, while pointing Marker DNA in the first well.

7) Connect the positive and negative poles to the electrophoresis tank and the electrophoresis instrument, turn on the power, set the time and voltage and start electrophoresis running.

8) After electrophoresis, remove the gel with gloves and transfer the gel to the gel imager to observe the results.

3.4 Purification of DNA

1) The gel containing the target fragment is cut out and transferred to an EP tube. The total weight is weighed on a balance and the Binding Buffer is added until the concentration reaches 0.1 mg/μL.

2) Place the EP tube in a water bath at 60°C. Turn the tube upside down every 2 min to dissolve the gel more rapidly until it is completely dissolved, then add the entire solution to the column.

3) Place in a centrifuge at 10,000r for 1min, discard the supernatant and repeat.

4) Add 300 μL of the binding buffer, level again in a centrifuge at 10,000 r and centrifuge for 1 min, discard the supernatant.

5) Add 700 μL Wash Buffer, level and centrifuge at 10,000 r for 1 min and repeat once.

6) Transfer the adsorbent column to a new EP tube and leave to stand for 2min.

7) Add 35μL ddH2O to the central band, level and centrifuge at 12,000r for 1min, discard the supernatant, then centrifuge again to obtain purified DNA.

3.5 Yeast transformation

1) Culture colonies for 12h, select yeast spots to be coated on plates and add fresh culture solution for 6h.

2) Transfer the yeast solution into a centrifuge tube, level in a centrifuge and centrifuge at 4000r for 2min, with the yeast centrifuged underneath the liquid.

3) Wash in water in an ultra clean table, discard the medium, add 5ml of dd water for internal use, level in a centrifuge and centrifuge at 4000r set for 2min.

4) After centrifugation the supernatant is removed and the dd water used is diluted tenfold.

5) Suspend with 1mL of 0.1M mol/L LiAc, dispense in an EP tube, mark with the bacterium number, level the EP tube in the centrifuge and centrifuge at 5000r for 1min.

6) Remove the supernatant with a pipette and add 240μl PEG and 36μl 1.0M mol/L LiAC.

7) Remove -25°C fish sperm DNA (ssDNA) in the fridge into boiling water for 5min and wait for it to cool in the water.

8) Transform PCR product fragment at -4°C, transfer to the bottom of an EP

tube and label.

9) Add 3.5μl of fish sperm DNA and mix in a vortexer until evenly distributed.

10) Incubate in a water bath at 30°C for 30min. then in a hot water bath at 42°C for 25min.

11) Transfer to a centrifuge for leveling and centrifuge at 5000r for 1min.

12) Remove the supernatant and coat the plates in SC-Ura-Nat.

13) After coating the plates are placed in a thermostatic incubator at 30°C to grow single colonies.

3.6 *E. coli* transformation

1) Place the sensory cell chamber on ice for 3 min, add 1 μL of plasmid to it and leave it on ice for 30 min.

2) Place in water at 42°C for 90 s and place on ice for another 3 min.

3) Add 1 μL of LB culture medium to it and transfer to a constant temperature incubator at 37°C for 1h.

4) At the end of the incubation the plates are coated (LB+100μLAmpicillin).

5) Afterwards place in incubator at 37°C and incubate for 24h.

6) After the growth of single colonies, a number of bacteria were picked out for verification.

3.7 Extraction of plasmids

1) Pick out a single colony and place it in 5mLLB+Amp medium and incubate at 37°C for 12-16 h at 200rmp.

2) Transfer the cultured bacteria into an EP tube, level it in a centrifuge, centrifuge at 12000r for 1min, discard the supernatant and collect all the bacteria.

3) Add 250 μL of Solution I (RNase I was added in advance and stored at 4°C in the refrigerator) and shake repeatedly to suspend the bacteria in the liquid.

4) Add 250 μL of Solution II to the solution and invert up and down several times (8-10 times) until the liquid becomes clear.

5) Add 350 μL of Solution III and invert up and down several times until a flocculent precipitate appears, centrifuge at 12000 rmp for 5 min.

6) Transfer the supernatant obtained after centrifugation to the collection tube of the adsorbed plasmid using a pipette, centrifuge at 12000 rmp for 1 min and discard the clear liquid in the collection tube.

7) Add 500 μL of Buffer HBC to this and centrifuge at 12000r for 1min.

8) Add 600 μL of DNA Wash Buffer, centrifuge at 12,000 r for 1 min and discard the clear solution from the collection tube, repeat at the end.

9) Continue centrifuging at 12,000r for 2min in the centrifuge.

10) Open the cap and leave the tube for 5min while maintaining the room temperature until the ethanol has evaporated completely.

11) Add 600μL of sterile water, leave for 2min, centrifuge at 12000r for

1min, discard the supernatant, add sterile water and centrifuge again at 12000r for 1min.

12) Finally, check the concentration of the plasmid.

3.8 Shaker fermentation

The brewer's yeast is transferred to a triangular flask containing the culture solution, placed in a shaker and fermented by shaking incubation in a shaker at a constant temperature of 30°C and incubated overnight.

3.9 5-Foa Discard Ura

3.9.1 Configuring the culture medium

Prepare the following media:

1 x 5-fluoroorotic acid medium, 1 x YEPD liquid medium, 1 x 200mL yeast complete synthesis medium (SC), 3 x 1L uracil-deficient complete synthesis medium (SC-URA), 1 x 400mL uracil-deficient complete synthesis medium (SC-URA).

3.9.2 Culture media

1) Start by adding 20mL of glucose to the YEPD medium.

2) Take 50mL of YEPD and add 100μL of antibiotics, shake about 20 times against.

3) Pour 25mL of SC-Ura-N into each of the two media, shake slightly, and

allow to dry.

4) Add another 250mL of EPD to 12.5mL of glucose and also add 500μL of antibiotics.

5) Pour one medium for every 25mL.

6) Allow to dry for 15min.

3.9.3 Picking out bacteria

Divide 16 compartments on each 5-Foa medium and paint four areas of the cultured colonies, with the outer circle delineated as a rectangle and the inner circle delineated as a triangle. After coating, place all the media in a 30°C incubator and incubate for 1 day.

3.9.4 Cell breaking

1) Implant the yeast into 100 ml and 200 ml of NaOH with 16 YEPD and 44 SC-Ura-N respectively.

2) Mix the mixture well.

3) After mixing, boil in a water bath for 10 min and leave at room temperature until room temperature.

4) After leveling in a centrifuge, centrifuge at 6000r for 1min.

Half of the cultured bacteria are coated, transferred to an EP tube and placed in a boiling pot for 10min.

5-FOA was transferred to a microwave oven to melt, cooled to about 50°C and transferred to an ultra clean table for extraction. Subsequently, 8ml of bacteria

were inoculated with YPD, shaken for 15h and then the 5-FOA was coated on the plate and colonies without Ura grew.

3.10 Measurement of growth curves

1) Do a pre-test by transferring the bacteria to an ultra-clean table that has been irradiated with UV light.

2) Adjust the concentration of the bacteria to be tested to 0.195-0.199Abs.

3) Divide the bacterial solution into 4 bottles and add 3.6g/mL, 3.8g/mL, 4.0g/mL and 4.2g/mL of acetic acid respectively.

4) Design a control solution of the same environment without acetic acid.

5) Transfer the brewer's yeast to a centrifuge and centrifuge at 4000r for 2min.

6) Measure the yeast concentration with a spectrophotometer and record at six hourly intervals.

7) After each measurement, transfer the triangular flask containing the yeast solution to a shaker to continue the proliferation.

8) Select the most appropriate concentration and design three parallel experiments to record the data.

9) Draw a growth curve of the yeast concentration after spotting.

Summary of the Chapter III

1. Each experimental step must be completed in an orderly manner.

2. The materials and instruments used in the experimental procedures need to be precise.

3. Each small step must be completed within the time limit and reagents and strains must be used appropriately to ensure that each step of the experiment is correct

CHAPTER IV
RESULTS AND ANALYSIS

4.1 H3K36Q point mutant construction scheme

4.1.1 H3K36Q point mutant construction process

Among chromatin modifications, lysine (Lys, K) modification is a common one and is essential for DNA replication, DNA repair, transcription and translation in living organisms. However, in the fermentation of brewer's yeast, the yeast is always exposed to acidic inhibitors such as acetic acid, which prolong the fermentation cycle. In order to improve the fermentation efficiency of *Saccharomyces cerevisiae*, the lysine at position 36 on histone H3 was modified with a point mutation to glutamine (Gln, Q) and then the mutated fragment was introduced into strain BSPZ001. The histone H3K36 point mutant strain of BSPZ001 was constructed as follows (Figure 4.1, Appendix A).

4.1.2 Amplification and transformation of DNA fragments

The HHT2 sequence of histone H3 was located and the HHT2 gene, which had been subjected to the H3K36 point mutation, was amplified by polymerase chain reaction PCR using primers P1 and P4, and the PCR product was subsequently removed for agarose gel electrophoresis (Figure 4.2, Appendix A).

The bands were observed in an electrophoresis gel viewer, using Marker DL5000 as a control, and bright bands were obtained, similar to the predicted length.

The gels were cut under UV light and placed in EP tubes, which were stored at -4°C.

4.1.3 Identification of positive clones using PCR

The gene for HHT2 containing the histone mutation in the recovered gum was transferred into strain BSPZ001 using PEG lithium acetate and the positive clone was screened by SC-Ura-N plates (Figure 4.3, Appendix B).

Rounded single colonies were selected on this SC-Ura-N plate.

These screened positive clones were painted on the SC-Ura-N and colonies were grown on the scribed lines (Figure 4.4, Appendix B). DNA from the scribed colonies was extracted and PCR amplification was performed using P6/Ura-2 and P8/Ura-3 as primers and the amplification products were used for gel electrophoresis, with MarkerDL5000 as control, yielding bright DNA bands of similar length to the predicted DNA, indicating that BSPZ001 had been transferred into the point mutant gene.

4.1.4 Screening for Ura-deficient histone mutant strains

Histone strains carrying the point mutation gene were coated onto plates of 5-FoA (Figure 4.5, Appendix C) for screening, and point mutant histone strains with loss of the Ura gene could be screened by the ability of 5-FoA to kill strains that synthesise uracil.

To determine this further, DNA was extracted from the strain and PCR amplified, and the amplified product was subjected to agarose gel electrophoresis (Figure 4.6, Appendix C), which under UV light showed that the DNA was a

segment less than its previous length, demonstrating that the mutant strain had lost Ura.

4.2 Acetic acid tolerance assay for the H3K36Q point mutant

4.2.1 Determination of the growth curve of the strain

In order to determine the acetic acid resistance of the point mutated brewer's yeast, the group needed to first measure the growth curve of the brewer's yeast in an acetic acid-free environment. The two types of brewer's yeast, spot mutated and unspot mutated, were first measured to form a control and propagated by pill bottle fermentation in a medium containing glucose and the growth curve was plotted as shown in (Figure 7).

The colonies were in the adjustment period in the first 4h and in the logarithmic growth period from 4 to 15h. There was no major difference in the rate between the punctured strain and the control, and after 15h the growth of the colonies was in the stable period without major changes, and the amount of punctured bacteria was slightly smaller than that of the control. Figure 4.7 (Appendix D) shows that histone H3K36Q point mutated *Saccharomyces cerevisiae* did not differ significantly from unpoint mutated *Saccharomyces cerevisiae* in the rate of multiplication in glucose-containing media.

4.2.2 Growth curve to detect acetic acid tolerance of the strain

The point mutated and unpoint mutated brewer's yeast were re-extracted and

transferred to a medium containing glucose and 3.8 g/L acetic acid to form a control. They were placed in a shaker for fermentation and growth curves were plotted as shown in (Figure 4.8, Appendix D).

The colonies without point mutation were in the adjustment period for the first 12h and grew exponentially after 12h; the colonies with point mutation were in the adjustment period for 24h and grew exponentially after 24h. Comparing the results of the two sets of experimental data showed that the brewer's yeast histone H3K36Q point mutation did not significantly enhance resistance to acetic acid compared to wild brewer's yeast.

Summary of the Chapter IV

1. First construct the H3K36Q point mutant strain and verify the full length, and within this process design primers to form the point mutant strain.

2. The mutated genes were transformed and amplified and then screened for positive clones using PCR techniques.

3. The strains were genetically discarded from Ura and screened, and the screened strains were used for.

4. The mutated strains were cultured and the growth curves in glucose, glucose and acetic acid were recorded at regular intervals

CONCLUSION

1. Acetylation modification of the H3K36 site in *Saccharomyces cerevisiae* in order to reduce the inhibitory effect of acetic acid produced by lignocellulose hydrolysis on the reproduction of *Saccharomyces cerevisiae*.

2. We successfully obtained the histone H3K36Q point mutation strain BSPZ001 by inserting a fragment of the H3K36Q point mutation gene of strain S288C into the genome of strain BSPZ001 by PCR, and constructed brewer's yeast that may have acetic acid resistance.

3. In the measured growth curves, we found that the growth rate of the point mutant *Saccharomyces cerevisiae* did not differ significantly from that of the wild type in glucose; in an environment with 3.8 g/L of acetic acid and glucose, the growth rate of the point mutant *Saccharomyces cerevisiae* did not increase significantly from that of the control wild type strain.

4. This result indicates that modification of the histone H3K36 locus has little effect on the acetic acid resistance of *Saccharomyces cerevisiae* and provides an experimental basis for further research into the mechanisms that increase the growth rate of *Saccharomyces cerevisiae*.

REFERENCE

1. 张雅琦. 利用木糖产乙醇酿酒酵母基因工程菌的构建及其代谢机制解析[D].北京化工大学,2022.DOI:10.26939/d.cnki.gbhgu.2022.001308.(Zhang Yaqi. Construction of ethanol-producing brewer's yeast genetically engineered using xylose and analysis of its metabolic mechanism[D]. Beijing University of Chemical Technology, 2022. DOI:10.26939/d.cnki.gbhgu.2022.001308.)

2. 陈玲玲,彭贵子,张伟丽等.突变在基因组进化中的意义[J].遗传 ,2006(05):631-638.DOI:10.16288/j.yczz.2006.05.023.(Chen L.L.,Peng G.Z.,Zhang W.L. et al. The significance of mutations in genome evolution[J]. Genetics,2006(05):631-638. doi:10.16288/j.yczz.2006.05.023.)

3. 吴永民, 葛建平.生物燃料乙醇产业研究综述与展望[J].安徽农业科学,2013,41(09):4008-4012.(Wu Yongxiang, Ge Jianping. A review and outlook on the creation of an ethanol industry for fuel [J]. Anhui Agricultural Science,2013,41(09):4008-4012.)

4. 郭孝孝,苗春雨,刘佳卉,姜明雪,田杨柳,张春雪,曲雪.小麦和玉米发酵制备乙醇生产工艺比较[J].中国酿造,2022,41(09):156-160.(Guo Xiaoxiao, Miao Chunyu, Liu Jiahui, Jiang Mingxue, Tian Yangliu, Zhang Chunxue, Qu Xue. Comparison of wheat and corn fermentation for ethanol production [J]. China Brewing,2022,41(09):156-160.)

5. ZABED H,SAHU J N,SUELY A,et al.Bioethanol production from rene wable sources:Current perspectives and technological progress[J].Renewable and S

ustainable Energy Reviews,2017,71:475-501.

6. Ma Y, Wang X R, Li Tong, et al. Hydrogen and ethanol:Production, stor age, and transportation[J]. International Journal of Hydrogen Energy, 2021, 46(54): 27330-27348.

7. ZABED H, SAHU J N, BOYCE A N, et al. Fuel ethanol production fro m lignocellulosic biomass:An overview on feed stocks and technological approach es[J]. Renewable&Sustainable Energy Reviews, 2016, 66:751-774.

8. AMBAYE T G,VACCARI M,BONILLA-PETRICIOLET A,et al.Emerging technologies for biofuel production:A critical rev iew on recent progress,challenges and perspectives[J].Journal of Environmental M anagement,2021,290:112627.

9. Ji X J, Huang H, Nie Z K, et al. Fuels and Chemicals from Hemicellulo se Sugars[J]. Biotechnology in ChinaIII： Biofuels and Bioenergy, 2012, 128:199-224.

10. 熊乙, 杨富裕, 倪奎奎,许庆方.微生物在木质纤维素降解中的应用进展[J].草学,2019(05):1-7.(Xiong Zhuang, Yang Fu, Ni Kui Kui, Xu Qing Yuan. Advances in the use of micromaterials in the degradation of woody cellulose[J]. Herbology,2019(05):1-7.)

11. 彭姿,谭兴和,熊兴耀等.木质纤维素糖化前预处理新技术研究进展[J].中国酿造,2013,32(01):1-4.(Peng Z, Tan XH, Xiong XY, et al. Research progress of new technology for pretreatment of lignocellulose before saccharification[J]. China Brewing,2013,32(01):1-4.)

12. 赵建志,李洪兴,王心凝等.适于二代燃料乙醇生产酿酒酵母的精准构制[J].生物产业技术,2018,No.66(04):74-83.(Zhao JZ, Li HX, Wang XN et al. Precise conformation of brewer's yeast suitable for second-generation fuel ethanol production[J]. Bioindustrial Technology,2018,No.66(04):74-83.)

13. SOUTO-MAIOR A M, RUNQUIST D, HAHN-HAGERDAL B.Crabtree-negative characteristics of recombinant xylose-utilizing *Saccharomyces cerevisiae*

14. 宁艳春，陈希海，王硕，等.纤维素乙醇研发现状与研究趋势分析[J].化工科技， 2020,28(1):65-68.(Ning Yanchun, Chen Xihai, Wang Shuo, et al. Analysis of the current status and research trends of cellulosic ethanol R&D[J]. Chemical Science and Technology, 2020,28(1):65-68.)

15. 瞿国凡, 姚春德, 吴涛阳, 王辉, 李壮壮, 闫俊杰.乙醇汽油与普通汽油非常规污染物排放特性对比研究[J].环境科学学报,2020,40(01):111-118.(Qu Guofan, Yao Chunde, Wu Taoyang, Wang Hui, Li Zhuangzhuang, Yan Junjie. Emission characteristics of non-conventional pollutants from ethanol gasoline and regular gasoline: a comparison[J]. Journal of Environmental Science,2020,40(01):111-118.)

16. FLETCHER E, KRIVORUCHKO A, NIELSEN J. Industrial systems bi ology and its impact on synthetic biology of yeast cell factories [J]. Biotechnology and Bioengineeing, 2016, 113(6): 1 164- 1 170.

17. 俞建良,熊强,林鑫,崔喜贵.新时代我国燃料乙醇产业发展新思路[J].中国酿造,2021,40(12):17-21.(Yu Jianliang, Xiong Qiang, Lin Xin, Cui Xigui. New

ideas for the development of China's fuel ethanol industry in the new era [J]. China Brewing,2021,40(12):17-21.)

18. 王定昌,赖荣婷.酵母的用途[J].粮油食品科技,2002(01):12-13+16.DOI:10.16210/j.cnki.1007-7561.2002.01.007.(Wang D C, Lai R T. The uses of yeast[J]. Cereal, Oil and Food Science and Technology, 2002(01):12-13+16. DOI:10.16210/j.cnki.1007-7561.2002.01.007.)

19. LU Z,PENG B,EBERT B E,et al.Auxin mediated protein depletion for metabolic engineering in terpene-producing yeast[J].Nat Commun,2021,12(1):1051.

20. PADDON C J,WESTFALL P J,PITERA D J,et al.High-level semi-synthetic production of the potent antimalarial artemisinin[J].Nature,2013,496(7446):528.

21. 于航, 周林, 袁鹏.中国燃料乙醇产业发展概况[J].粮食与食品工业,2009,16(04):34-37.(Yu Hang, Zhou Lin, Yuan Peng. An overview of the development of the fuel ethanol industry in China[J]. Food and Beverage Industry, 2009,16(04):34-37.)

22. SHI Y,WANG D,LI R,et al.Engineering yeast subcellular compartments for increased production of the lipophilic natural products ginsenosides[J].Metab Eng,2021,67:104.

23. 赵小丽,甄玉国,王兰惠等.酿酒酵母发酵条件的研究[J].中国酿造,2014,33(06):75-78.(Zhao S. L., Zhen Y. G., Wang L. H. et al. Study on the fermentation conditions of brewer's yeast[J]. China Brewing,2014,33(06):75-78.)

24. ABO B O,GAO M,WANG Y,et al. Lignocellulosic biomass for bioethanol:An overview on pretreatment,hydrolysis and fermentation processes[J]. Rev Environ Health,2019,34(1):57-68.

25. ROBAK K,BALCEREK M. Review of second generation bioethanol production from residual biomass[J]. Food Technol Biotechnol,2018,56(2):174-187.

26. 祝涛,李少白,王瑶.木质纤维素乙醇原料预处理技术的研究进展[J].广东化工,2013,40(17):108-109+111.(Zhu Tao, Li Shaobai, Wang Yao. Research progress of pretreatment technology for lignocellulosic ethanol feedstock[J]. Guangdong Chemical Industry,2013,40(17):108-109+111.)

27. Danuza Nogueira Moysés;;Viviane Castelo Branco Reis;;Jo?o Ricardo Moreira de Almeida;;Lidia Maria Pepe de Moraes;;Fernando Araripe Gon?alves Torres.Xylose Fermentation by *Saccharomyces cerevisiae*: Challenges and Prospects[J].International Journal of Molecular Sciences,2016(3).

28. GOFFEAU A,BARRELL B G,BUSSEY H,et al.Life with 6000 genes[J].Science,1996,274(5287):546,563-567.

29. CHUNDAWAT S P S, BECKHAM G T, HIMMEL M E, et al. Deconstruction of lignocellulosic biomass to fuels and chemicals[J]. Annu Rev Chem Biomol Eng, 2011, 2:121-145.

30. KIM D. Physico-chemical conversion of lignocellulose:Inhibitor effects and detoxification strategies:A mini review[J]. Molecules,2018,23(2):309.

31. Casal M, Cardoso H, Leao C. Mechanisms regulating the transport of acetic acid in *Saccharomyces cerevisiae* [J]. Microbiology-Uk, 1996, 142: 1385-1390.

32. 赵景龙，张红霞，张秀红.低酸环境对汾酒酿造的影响[J].中国酿造，2013,32(8):35-38.(Zhao Jinglong, Zhang Hongxia, Zhang Xiuhong. Influence of low acid environment on Fenjiu brewing[J]. China Brewing, 2013,32(8):35-38.)

33. KUMAR V,YADAV S K,KUMAR J,et al. A critical review on current s trategies and trends employed for removal of inhibitors and toxic materials generat ed during biomass pretreatment[J]. Bioresour Technol,2020,299:122633.

34. 刘兴艳,贾博,赵芳等.酿酒酵母对弱有机酸胁迫的应激机制研究进展[J]. 食品与发酵工业,2013,39(06):125-129.DOI:10.13995/j.cnki.11-1802/ts.2013.06.030.(Liu XY, Jia B, Zhao F et al. Research progress on the stress mechanism of *Saccharomyces cerevisiae* in response to weak organic acid stress[J]. Food and Fermentation Industry,2013,39(06):125-129.doi:10.13995/j.cnki.11-1802/ts.2013.06.030.)

35. 胡丹,周希贵,胡炎华,李岩.L-苏氨酸发酵过程中乙酸的控制[J].发酵科技通讯,2012,41(04):4-8.DOI:10.16774/j.cnki.issn.1674-2214.2012.04.007.(Hu D,Zhou XG,Hu YH,Li Y. Control of acetic acid during L-threonine fermentation[J]. Fermentation Science and Technology Letters,2012,41(04):4-8.DOI:10.16774/j.cnki.issn.1674-2214.2012.04.007.)

36. 耿鹏. 酿酒酵母乙酸耐受基因的定位、筛选及初步验证[D].江南大学,2017.（Geng Peng. Localization, screening and preliminary validation of acetic

acid tolerance genes in *Saccharomyces cerevisiae* [D]. Jiangnan University,2017.)

37. 程国平,陈琛,李必金等.工业微生物乙酸响应与调控机制的研究进展[J]. 发酵科技通讯,2020,49(04):230-235.DOI:10.16774/j.cnki.issn.1674-2214.2020.04.010.(Cheng G. P., Chen C., Li B. J. et al. Research progress on the response and regulatory mechanism of industrial microbial acetic acid[J]. Fermentation Science and Technology Letters,2020,49(04):230-235.DOI:10.16774/j.cnki.issn.1674-2214.2020.04.010.)

38. CASEY E,SEDLAK M,HO N W,et al.Effect of acetic acid and pH on th e cofermentation of glucose and xylose to ethanol by a genetically engineered strai n of *Saccharomyces cerevisiae*[J].FEMS yeast research,2010,10(4):385-393.

39. Thoma F, Koller T, Klug A. Involvement of histone H1 in the organizati on of the nucleosome andof the saltdependent superstructures of chromatin[J].Jour nal of Cell Biology,1979,83: 403－427

40. 茅卫锋,汪亚平,孙永华,吴刚,陈尚萍,朱作言.虹鳟鱼(Oncorhynchus mykiss)组蛋白 H_3 启动子的分子克隆及在稀有(鱼句)鮈(Gobiocypris rarus)中的表达活性分析[J].自然科学进展,2004(01):48-52+123.(MAO Wei-Feng, WANG Ya-Ping, SUN Yong-Hua, WU Gang, CHEN Shang-Ping, ZHU Zuoyin. Molecular cloning of the histone H_3 promoter from rainbow trout (Oncorhynchus mykiss) and analysis of its expression activity in the rare (fish sentence) carp (Gobiocypris rarus)[J]. Advances in Natural Sciences,2004(01):48-52+123.)

41. 张静,苗佳敏,李玉珠等.植物组蛋白 H3 的研究进展[J].草原与草坪 ,2022,42(04):147-157.DOI:10.13817/j.cnki.cyycp.2022.04.019.(ZHANG

Jing,MIAO Jia-Min,LI Yu-Zhu et al. Research progress of plant histone H3[J]. Grassland and Turf,2022,42(04):147-157.DOI:10.13817/j.cnki.cyycp.2022.04.019.)

42. TESSARZ P,KOUZARIDES T. Histone core modifications regulating nucleosome structure and dynamics[J]. Nat Rev Mol Cell Biol,2014,15(11):703-708.

43. Ruthenburg AJ et al.Multivalent engagement of chromatin modifications by linked binding modules.Nat Rev Mol Cell Biol,2007,8:983-994

44. Jenuwein T et al.Translating the histone code.Science,2001,293:1074-1080

45. Kouzarides T.Chromatin modifications and their function.Cell,2007,128(4):693 ~ 705

46. TESSARZ P,KOUZARIDES T. Histone core modifications regulating nucleosome structure and dynamics[J]. Nat Rev Mol Cell Biol,2014,15(11):703-708.

47. 周蔚,王世鑫.组蛋白修饰与基因调控[J].国外医学: 分子生物学分册,2003,25(2):71 － 73.(Zhou W, Wang S-X. Histone modifications and gene regulation[J]. Foreign Medicine: Molecular Biology, 2003,25(2):71-73.)

48. 郭晓强.组蛋白修饰研究的历程和意义[J].科学,2017,69(02):37-41.(Guo Xiaoqiang. The history and significance of histone modification research[J]. Science,2017,69(02):37-41.)

49. Dutta A, Abmayr SM, Workman JL. Diverse Activities of Histone Acylations Connect Met

abolism to Chromatin Function[J]. Mol Cell.2016;63(4):547-552.

50. 陈攀峰,丰继华,单增辉等.酵母基因组组蛋白修饰之间相关性的研究

[J].基因组.(Chen Panfeng, Feng Jihua, Shan Zenghui et al. Correlation between histone modifications in the yeast genome[J]. Genome.)

Appendix A

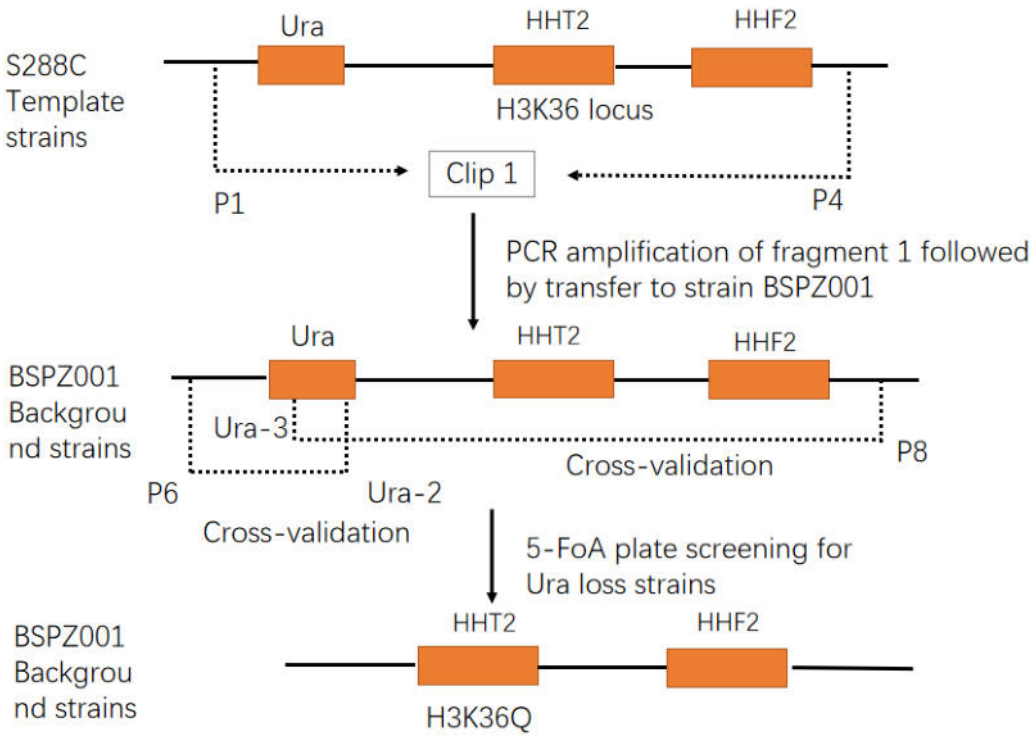

Figure 4.1 Obtaining BSPZ001 strains with histone point mutations

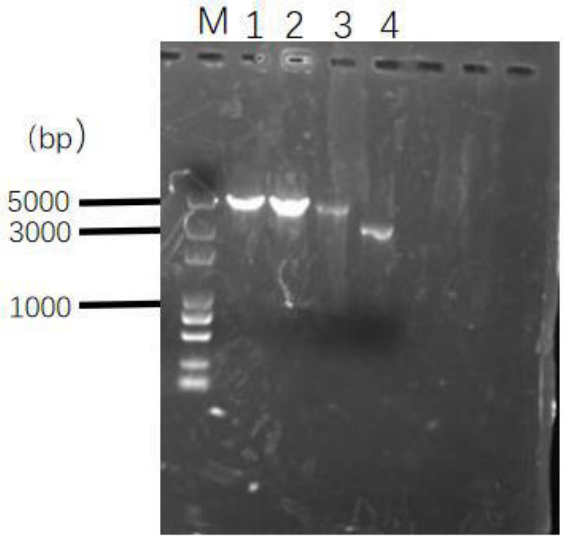

Figure 4.2 Electropherogram of PCR products，M：Marker

Appendix B

Figure 4.3 Transformed positive clone of H3K36Q yeast

Figure 4.4 Delineation of positive clone DNA purification

Appendix C

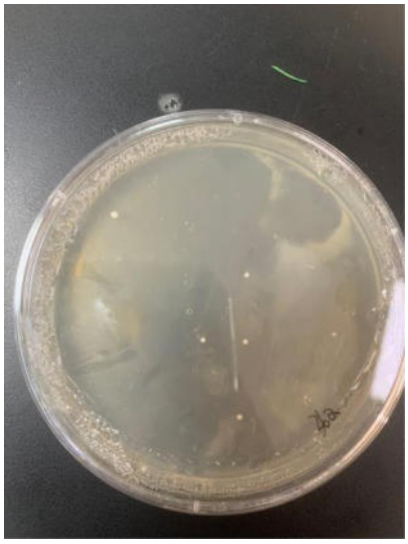

Figure 4.5 Histone strains growth onto plates of 5-FoA

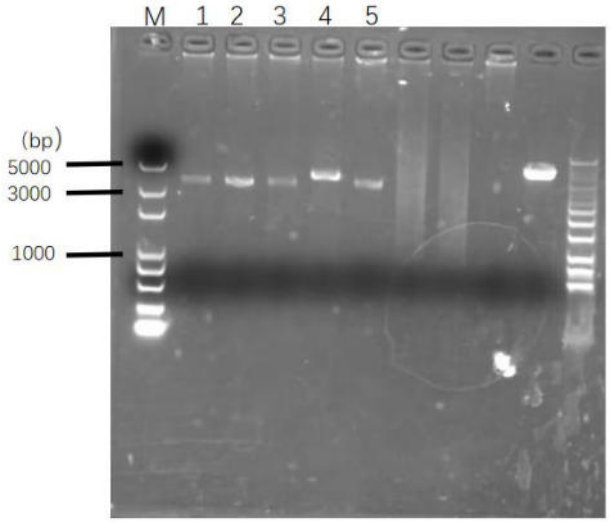

Figure 4.6 Agarose gel electrophoresis

Appendix D

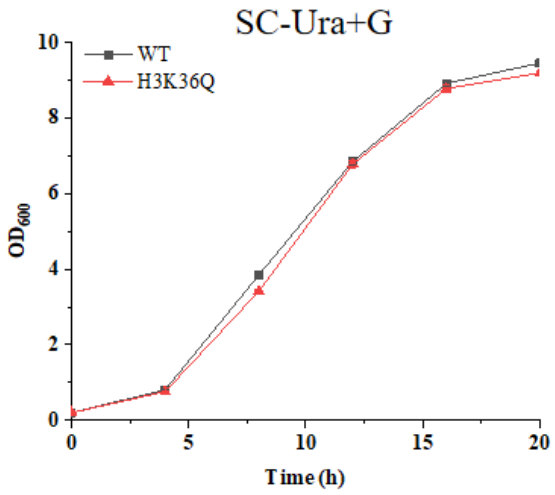

Figure 4.7 Brewer's yeast growth curve WT: Control

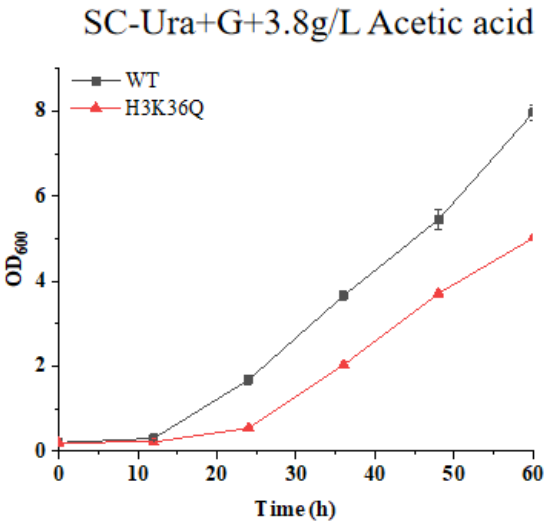

Figure.4.8 Brewer's yeast growth curve in acetic acid environment