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 Separation and screening of Acetobacter producing alcohol dehydrogenase

Completed: student of group BEBT-19
of specialty 162 «Biotechnology and Bioengineering»
Haonan DONG 董昊男
(Student's First and LAST NAME)

Scientific supervisor Olga Stepdlovska
(first name, LAST NAME)

Reviewer Ihor Hretskyi
(first name, LAST NAME)

Kyiv 2023

35

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
Haonan DONG
(Student's Full Name)

1. Thesis topic Separation and screening of Acetobacter producing alcohol dehydrogenase

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 data about alcohol dehydrogenase characte. and obtaining experimental data about isolation and screening Acetobacter sp. with high ADH activity

4. Content of the thesis (list of questions to be developed): literature review, experimental materials and experimental methods, experimental results and discussion

5. Date of issuance of the assignments 01.05.23

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	董昊男
2	Section 1	05.05.23	董昊男
3	Section 2	07.05.23	董昊男
4	Section 3	11.05.23	董昊男
5	Conclusions	13.05.23	董昊男
6	Draw up a bachelor's thesis (final version)	14.05.23	董昊男
7	Submission of bachelor's thesis to the department for review (14 days before the defense)	15.05.23	董昊男
8	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	27.05.2023	2% - 22% 董昊男
9	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	30.05.23	董昊男

Student 董昊男 (signature) Haonan DONG (first name, LAST NAME)

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

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

ABSTRACT

In mammals, alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) constitute the main oxidation channels of ethanol metabolism, which are the main limiting factors of ethanol metabolism in the body. ADH mainly catalyzes the production of acetaldehyde from ethanol and is the first step in the oxidized process of ethanol in various organs and tissues of humans and animals. Therefore, it is of great significance in relieving alcoholism and preventing liver damage caused by ethanol. Alcohol dehydrogenase has been found in animals, plants, microorganisms, eukaryotes, and prokaryotic bacteria, which is widely presented in microorganisms. Modern research has proven that the brewing of vinegar is carried out by cell membranes of acetic acid bacteria binding enzymes that complete acetic acid fermentation through the oxidation of alcohol-by-alcohol dehydrogenase and the oxidation of acetaldehyde-by-acetaldehyde dehydrogenase.

The fermentative ability of acetic acid bacteria is related to the activity of these two enzymes, especially the former. Therefore, isolating strains with high ADH production from vinegar is of great research which is significant for establishing a probiotic library for detoxifying alcohol and protecting the liver. This article enriched and screened well-known vinegar mash samples in China, and obtained *Acetobacter* D2 with ADH activity reaching 4.288×10^{-2}U. Furthermore, its activity was improved to 8.254×10^{-2}U through UV mutagenesis.

The object of the work is *Acetobacter* D2 with ADH activity. The subject of the work is ADH activity of isolated from vinegar *Acetobacter* D2.

The aim of the work is screen and isolate effective *Acetobacter* produced alcohol dehydrogenase. The tasks of the work are to isolate from vinegar ADH active strain of *Acetobacter*, obtain the ADH activity, and improve ADH activity with UV mutagenesis.

Key words: Alcohol dehydrogenase; Acetobacter; Screening; UV mutagenesis; enzyme.

CONTENT

INTRODUCTION

Acetobacter is a genus of bacteria that can be involved in various biological processes, including the production of acetic acid during fermentation. However, it's important to note that alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) are enzymes that are more commonly associated with the metabolism of alcohol in humans and some other organisms, rather than Acetobacter.

ADH is responsible for the conversion of alcohol (ethanol) to acetaldehyde, while ALDH converts acetaldehyde into acetic acid. These enzymes are crucial in the breakdown of alcohol in the body, specifically in the liver. They play a vital role in alcohol metabolism and the detoxification of acetaldehyde, which is a toxic compound.

In acetobacter, the main enzyme involved in the conversion of ethanol to acetic acid is ethanol dehydrogenase (EDH), rather than ADH. EDH catalyzes the oxidation of ethanol to acetaldehyde, which is then further oxidized to acetic acid by acetaldehyde dehydrogenase. This process is an essential step in the production of vinegar, as acetobacter bacteria convert ethanol to acetic acid through these enzymatic reactions.

So, while acetobacter bacteria do possess enzymes involved in the oxidation of ethanol, they are not specifically referred to as alcohol dehydrogenase and acetaldehyde dehydrogenase like in humans and other organisms. Instead, they have their own specific enzymes for the conversion of ethanol to acetic acid.

CHAPTER I
LITERATURE REVIEW

1.1 Introduction to the ADH

Ethanol dehydrogenase (ADH) is a zinc containing enzyme, which Can catalyze the oxidation of ethanol into acetaldehyde and the coenzyme NADH. Ethanol-producing dehydrogenases are found in many microorganisms, including certain bacteria, fungi, etc. With a wide substrate specificity range (with high affinity and selectivity for specific substrates), it belongs to the first subclass of oxidoreductases. As a key enzyme in the metabolism of short-chain alcohol in organisms, it plays a very important catalytic role. ADH is an intracellular enzyme, and it also exists in cells of animal and plant. Both Ethanol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) are the main enzymes in the pathway of ethanol metabolism. Alcohol dehydrogenase transforms ethanol to acetaldehyde, while acetaldehyde dehydrogenase further converts acetaldehyde to acetic acid

The main single chain alcohol metabolism is the main speed limiting factor of ethanol metabolism in the body, so ADH is needed to convert ethanol into acetaldehyde enzyme, from a medical point of views, ethanol dehydrogenase has important significance to human, which has been widely used in medical field, such as the preparation of anti-alcohol drugs and determine the amount of alcohol methods etc. Moreover, ethanol dehydrogenase can be used for the detection of ADH in the human liver and it is important to prevent hangover and ethanol-induced liver injury. In addition, ethanol can be used for microbial fermentation. The aroma molecules of some artificial spices are also produced by ADH by oxidizing alcohol in fruits, herbs and flowers.

1.2 Research status of ADH application

ADH has catalytic characteristics, and it has a corresponding role in science, brewing and other fields. People can use its characteristics to make biosensors. It can be said that the technology in this aspect has entered the mature stage. In addition,There are currently several aspects of research on ADH.

1.2.1 Bioelectrode and ethanol sensor

Bioelectrodes are electrochemical sensors that design and construct using biological architecture and function. The ethanol sensor is a bioelectrode capable of measuring the ethanol concentration. It uses ethanol dehydrogenase (ADH) as a bioelectrocatalyst to convert the concentration of ethanol into electrical signal output. Such sensors have the advantages of fast, accurate, reproducible, good selectivity, and high sensitivity, so they are widely used in industry, medical, and life sciences. The most important part of the ethanol sensor preparation process is the preparation of the bioelectrodes. There are three types of common biological electrodes: enzyme-based electrodes, Cell electrodes, and tissue electrodes. Among these, enzyme-based electrodes are the most commonly used one. There are many methods of preparing ethanol sensors, and the common method is to fix the enzyme or cell on the electrode surface. During the preparation, our attention is needed to select the appropriate enzyme, materials and reaction conditions to optimize the sensitivity and selectivity of the sensor. The preparation process of sensor generally includes the preparation of biological electrode, sensor assembly, sensor testing and data processing etc. In the preparation of bioelectrodes, ethanol dehydrogenase was immobilized on the electrode surface to form an ethanol dehydrogenase electrode. In the assembly of the sensor, the electrode is connected to the electronic circuit to form the complete sensor. In the test of the sensor, different concentrations of ethanol solution are added to the sensor, and the size of the electrical signal is measured to obtain the ethanol concentration data, calculate and analyze the data to obtain the final ethanol concentration results. Ethanol

sensors are widely used in alcohol production, medical diagnosis, environmental monitoring, and food safety. In alcohol production, ethanol sensors can be used to monitor the alcohol concentration and ensure the quality and taste of the wine. In medical diagnosis, ethanol sensors can be used to detect ethanol concentrations in the blood and help doctors diagnose and treat diseases such as alcohol poisoning, and to detect ethanol concentrations in the driver's blood and determine problems such as drunk driving. In environmental monitoring, ethanol sensors can be used to monitor the ethanol concentration in the air and assess the environmental risks of industrial production. In food safety, ethanol sensors can be used to detect the ethanol content in food to ensure the quality and safety of food.

1.2.2 Application of ADH in industry

Ethanol dehydrogenase (ADH) is an enzyme that catalyzes the oxidation of ethanol to acetaldehyde and plays a very important role in green chemistry due to its efficient catalysis. However, ADH is less stable in the free state and is often limited in practical applications. Therefore, scientists work to find ways to stabilize this enzyme to facilitate its development. A commonly used approach is using vehicle to fix ethanol dehydrogenase in order to improve its stability and loss of activity. Gelatin is a commonly used carrier to immobilize ethanol dehydrogenase by cross-linking before preparation, which greatly improves the catalytic efficiency of the enzyme. In addition, many other carriers, such as nanoparticles, magnetic microbeads, are also used to immobilize ethanol dehydrogenase to improve its stability and activity. ADH also plays an important role in ethanol production, and using genetic engineering techniques can greatly improve the ethanol yield. For example, Lu Jian et al. constructed a genetically engineered strain with directional function, where glucose and xylose produced 21 and 5 times higher yields, respectively.

1.2.3 Application of ADH in food and drug fields

Moderate drinking is conducive to health, long-term drinking or heavy drinking will damage the nerve center, light nausea and vomiting, heavy lead to life threat. The World Health Organization shows that the global traffic accidents caused by drunk driving account for 50~60%. In recent years, people have also been aware of the harm of wine, and began to focus on the decomposition process of wine. Through the initial decomposition of the wine in the stomach (this process is metabolized) process understanding, ADH acts as a catalyst that can be used for the synthesis of specific drug molecules. These drugs can be directed designed to be exploited through metabolic pathways after entering the human body to achieve the desired potency. Liquants are currently in the United States**Ошибка! Источник ссылки не найден.** The field is developing rapidly, and its products can deliver ADH into the body through oral administration, which can increase the concentration of ADH in the body, and ultimately improve the efficiency of alcohol solution.

Ethanol dehydrogenases are enzymes that catalyze the conversion of ethanol to acetaldehyde. In the food industry, ethanol dehydrogenase is widely used in the brewing and alcoholic beverage production process to promote the fermentation of alcohol and improve the alcohol of products. Specifically, ethanol dehydrogenase can help brewers better control the amount of alcohol produced during the brewing process, thus affecting the taste and quality of the final product.

When brewing beer, the brewer can add a certain amount of ethanol dehydrogenase to promote the conversion of glucose in the wort, thus realizing the alcohol content and taste control of the beer. In the wine brewing process, ethanol dehydrogenase can help the wine fermentation speed faster and more stably, thus improving the product quality and yield. In addition, ethanol dehydrogenase can also be applied in the production of food additives and flavoring agents. For example, some beverages and tobacco products contain natural and artificial spices added with ethanol dehydrogenase to enhance their taste and aroma. Artificial

spices are usually made chemically together, but they also contain many natural substances, such as fruits, herbs and flowers. Some of these natural substances contain alcohol, requiring the use of ADH to oxidiit to acetaldehyde, creating more complex aroma molecules. In industrial production, ADH is usually extracted from yeast or bacteria and then used to make artificial spices. In conclusion, ethanol dehydrogenase has wide applications in the food industry and can provide more choices and better product quality control means for food producers. In addition, ADH also plays an important role in the growth and development of animals and plants. ADH also has important applications in the field of diet biology.

1.2.4 Applications in the biological sciences

For ethanol metabolism, ADH oxidizes ethanol to acetaldehyde and integrates NAD^+Reduction to the NADH. This process is the primary pathway for ethanol metabolism in many organisms. In humans, ADH is mainly found in the liver and stomach, so the study of ethanol metabolism is mainly focused on these two organs. By studying the expression and function of ADH in these organs, we can better understand the biological mechanisms of ethanol metabolism, thus providing new ideas and approaches for the treatment of ethanol metabolism-related diseases.

In addition, ADH also much attention in human gene polymorphism. There are seven alleles in the human ADH gene (ADH 1 A, ADH 1 B, ADH 1 C, ADH 4, ADH 5, ADH 6 and ADH 7), and polymorphisms in these genes are closely related to differences in ethanol metabolism.

For example, the ADH 1 B * 2 allele is more prevalent among Asians, and this gene is associated with faster rates of ethanol metabolism and is therefore important for the regulation of ethanol metabolism and differences in ethanol metabolic capacity between individuals. In some studies, the ADH gene polymorphism has also been closely related to the occurrence of ethanol-related diseases, such as alcohol abuse and alcoholism.

Overall, ADH has extensive research value in the field of biological sciences and genetic polymorphisms. As technology continues to evolve and research deepens, we believe that more important discoveries will emerge.

1.2.5 Application in bioengineering research

ADH is widely found in various organisms and can catalyze the conversion reaction of alcohols to aldehydes. ADH is widely used in the field of bioengineering, which can be discussed from the aspects of basic scientific research, medical chemistry, diet biology, environmental protection and so on. In basic science research, ADH is an important research object. By targeting the ADH isoenzyme gene polymorphism.

The in-depth study of ADH enzyme activity and structure is helpful to better understand its role in the biological metabolic process and the related issues in life science. For example, studying the structure and function of ADH can help us better understand its role in metabolic processes and the therapeutic effects of specific inhibitors targeting the structure of ADH enzymes for some diseases. Moreover, exploration based on ADH enzyme activity can provide a better understanding of biological metabolic processes and provide an important basis for the regulation of metabolic pathways.

1.3 Introduction to the ADH-producing strains

The ADH of organisms is generally dimeric, with a few being tetraploid [1]. SsADH is an ancient bacterial enzyme of the medium chain ADH family, and its three-dimensional structure has been deeply understood by scientists. ADH producing strains refer to strains that can produce ethanol dehydrogenase (ADH), and there are many types, including yeast, lactic acid bacteria, thermophilic bacteria, etc.

The isolation and screening of ADH producing strains are usually carried out through laboratory technology. The commonly used method is to screen bacterial

communities using substrates sensitive to ADH, such as alcohol or acetaldehyde. Identify the genomic information of ADH producing strains through genomics research to determine their characteristics and functions. By studying the characteristics and functions of these strains, their biocatalytic effects can be better utilized to promote the development of production and environmental protection.

1.3.1 Overview of *Acetobacter* sp

Acetobacterium (*Acetobacter*) is a Gram-negative bacteria that usually live in oxygen-rich environments, including wine, vinegar, fruit, flowers, and soil. And acetetobacter is a typical acetate fermentation bacteria, its metabolic pathway can oxidize ethanol to acetic acid, and produce a large amount of energy. *Acetobacter* is characterized by a high degree of redox capacity and acetic acid tolerance. Its cell walls are mainly composed of polysaccharides and proteins, with certain toughness and elasticity, allowing them to survive and reproduce in an oxygen-enriched environment. In addition, they can also be used in environmental governance areas such as biological control and remediation of soil pollution. *Acetobacter* are also an important model organism in molecular biology studies because they have certain genome stability and easy manipulation, and are one of the ideal model organisms for studying gene expression and metabolic pathways. The genome size of *Acetobacter*, about 3-4 Mbp, with high GC content and relatively stable gene number and structure, can be used to study biological issues such as gene expression, metabolic pathways and cell signaling. Furthermore, the genome of *Acetobacter* has been fully sequenced, including plasmids and chimeric vectors available for gene conversion and gene expression, etc. *Acetobacter*ia form an indefinite rod, typically 0.3 – 1.0 μm×1.0-3.0 μ m, with unipolar or bipolar flagella, and capable of moving in an oxygen-rich environment at a rapid rate. In addition, *Acetobacter* can also form cell groups and colonies, which can appear yellow, red, brown and other colors on its surface. The metabolic pathways of *Acetobacter* are very diverse, including sugar metabolism, acid metabolism, fatty

acid metabolism and nitrogen metabolism. The most typical one is the metabolic pathway of ethanol oxidation to acetic acid, which is one of the reasons why *Acetobacter* plays an important role in vinegar production. This metabolic pathway mainly includes the action of ethanol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH) and acetate dehydrogenase (ACDH).

In addition to its applications in the food industry, environmental governance and molecular biology research, *Acetobacter* has some other applications, such as the production of plastics and biofuels, which can produce acetic acid and polyhydroxyalkane ester compounds through the metabolic pathway, with potential applications.

1.3.2 Application and research status of *Acetobacter* sp

At present, people's research on acetic acid has penetrated into various fields, and *Acetobacterium* has a wide range of applications and value in food industry, biomedicine, skin care, environmental governance, ecological agriculture, synthetic biology and other fields. With the continuous progress of science and technology and the continuous expansion of its application, the application prospect of *Acetobacter* will continue to expand and deepen.

With people's attention to health and the pursuit of traditional food, the market demand for natural fermented food and vinegar products is also growing, as the key microorganism in the production of vinegar and other natural fermented food, it is expected to gain more extensive application and commercial value in the future market competition.

Acetobacterium is the key microorganism in vinegar making, which can ferment alcohol into acetic acid. Its fermentation products are not only widely used in condiments and food additives, but also have the functions of antibacterial, preservation and acid increase, which can extend the shelf life of food, so it is widely used in the food industry.

In addition to making vinegar, *Acetobacter* can also be used in the

production of naturally fermented foods. For example, the application of *Acetobacter* and other microorganisms such as yeast and Streptococcus thermophilus to make natural fermented foods such as yogurt, pickled cabbage and pickles, can not only improve the taste and nutritional value of the food, but also increase the content of its probiotics, which is beneficial to human health.

Acetobacter is also widely used in medical diagnosis. *Acetobacterium* can produce acetaldehyde by oxidized ethanol to turn the acetaldehyde on the medium to red, a property applied to the diagnosis of tuberculosis. In the diagnosis of tuberculosis, doctors can use urine, sputum and other samples to determine whether the patient is infected with tuberculosis bacteria by cultivating *Acetobacter*ia and observing the color change of acetaldehyde on the culture medium.

Acetobacter can use cholic acid and bile salt as a carbon source and energy source to synthesize deoxycholic acid through the metabolic pathway. *Acetobacter* hydrolyzes bile salts by bile salt hydrolase, breaking them into bile acids and choline. Then, cholic acid undergoes through a series of metabolic reactions, which is converted into methylmalonic acid, formic acid, pyruvate and other compounds, and then is synthesized as deoxycholic acid.

Deoxycholic acid is a bile acid derivative with various biological activities, including antibacterial, antiviral, cholesterol lowering and other biological applications in medicine and health care. The synthesis of deoxycholic acid through the metabolic pathway of *Acetobacter* can not only achieve the mass production of deoxycholic acid, but also control the synthesis efficiency and quality of deoxycholic acid by regulating its metabolic pathway.

Since *Acetobacter* belongs to the family Lactobacilli (*Acetobacteraceae*), it can grow and reproduce in a variety of environments, but the most suitable conditions for growth are 25-30℃ and pH 4.5-6.0, Enough oxygen. Under such conditions, *Acetobacter* can produce acetic acid through oxidized ethanol or other carbon sources for metabolism and growth. In practical application, the growth

conditions and media composition of *Acetobacteria* are usually adjusted, in order to improve the efficiency of acetic acid production.

For example, adjusting the temperature and pH allows *Acetobacter* to grow and produce acid in different environments. Adjusting the ratio of carbon and nitrogen sources can also affect the metabolism and acid production efficiency of *Acetobacter*. In addition, there are some auxiliary culture conditions, such as ventilation, and agitation, which can also affect the growth and metabolism of acetobacilli.

1.3.3 Feasibility of ADH production by *Acetobacter* sp

Acetobacter itself can produce ethanol dehydrogenase. Alcohol dehydrogenase is an essential enzyme during their metabolism, so they naturally produce this enzyme. For example, the large-scale application of *Acetobacterium* in the vinegar industry can produce acetic acid through organic matter, because the ethanol dehydrogenase and acetaldehyde dehydrogenase in its membrane work together, so that the organic layer are degraded to get acetic acid. Studies have found that ADH is mostly located in the cell membrane, and its amount and activity will be correspondingly increased under the action of alcohol induction. Therefore, we can use this property to explore under which conditions *Acetobacter* reproduces the fastest and produces the most ADH, and then screen to obtain the target strain. In addition, *Acetobacter* is a very easy to obtain bacterial species, which provides great convenience for the development of the experiment, and the experimental method is simple, and it has a high feasibility.

1.4 Research content and significance of this paper

1.4.1 The content of the study

First, the fermented vinegar grains were diluted and enriched, and the cultured strains were transferred into solid medium containing different alcohol concentrations to continue coating and culture. The growing colonies were picked for single colony crossed culture, and the single colonies with strong growth capacity were selected from the crossed culture for enrichment culture. Then, the activity of each ethanol dehydrogenase and the activity of ADH were measured, and the strains with high activity were identified morphologically and biochemically to confirm the selected *Acetobacteria*. We then further obtained the mutant strain with higher ADH activity.

1.4.2 The significance of the study

After we drink alcohol, about 10-20% of the alcohol is absorbed through the stomach and 75-80% in the small intestine. Most (90%) of the alcohol absorbed through the stomach is metabolized through the liver, and the rest is excreted through breath and urine, about 5% each. Alcohol is metabolized in the liver, first from ethanol (alcohol) to acetaldehyde under the action of ethanol dehydrogenase, acetaldehyde to acetic acid under the action of acetaldehyde dehydrogenase, and finally to carbon dioxide and water discharged from the body.

In mammals, ethanol dehydrogenase (Alcohol dehydrogenase, ADH) and aldehyde dehydrogenase (Acetaldehyde dehydrogenase, ALDH) constitute the main oxidation channel of ethanol metabolism, is the main rate limiting factor of ethanol metabolism in the body, ADH mainly catalytic ethanol to produce acetaldehyde, is the first step in human and animal organs and tissues, therefore in alcohol and prevent liver damage caused by ethanol.

Alcohol dehydrogenases have been found in animals, plants, microorganisms, eukaryotes, and prokaryotic bacteria, and they are widely found

in microorganisms. Modern studies have proved that the brewing of vinegar is performed by the cell membrane-bound enzyme by ethanol dehydrogenase (ADH) that oxidizes acetaldehyde and acetaldehyde dehydrogenase (ALDH) which reoxidizes acetaldehyde to acetic acid. The fermentation capacity of acetic acid bacteria is related to the activities of these 2 enzymes, especially the former. Therefore, the isolation of high-yield ADH strains from vinegar is important for the establishment of a probiotic library for liver protection.

CHAPTER Ⅱ

EXPERIMENTAL MATERIALS AND EXPERIMENTAL METHODS

2.1 Experimental reagents and consumables

Four different grains of fermented vinegar were classified as (A, B, C, D), glucose, yeast powder, ethanol, agar powder, calcium carbonate, absolute ethanol, and distilled water.

2.2 Main experimental instruments

Shaaker, incubator, constant temperature water bath, vibration shaker, pipetting gun.

2.3 Culture medium and buffer solution

Sugar-free medium: (5 portions and 25 mL each): 1g of yeast powder, 2 mL of ethanol. Sugar-free liquid medium (with 100 mL solution): 1 g yeast powder, 2.5 g agar, 1.5 g calcium carbonate, and absolute ethanol in different amounts as required.

The above medium was first sterilized in an autoclave cooker for 115℃, 30 min, and then cooled to about 40℃.

2.4 Experimental Methods

2.4.1 Enriched culture

1ml of vinegar fermented grains samples were absorbed with a pipette gun and added to a triangular bottle containing 10 mL sterile water containing sterilized glass beads with shaking. Then, 2 mL of supernatant was absorbed into sugar-free liquid culture medium, which was then placed in a constant temperature shaker for further cultivation. The temperature was controlled at 37℃, the speed was 160 r/min, and incubated for 36 h.

2.4.2 Isolation and purification

1 mL was absorbed from the enriched culture medium and moved to sugar-free solid medium containing 0%, 1%, 2%, 3%, 4% and 5% ethanol, respectively. It was applied evenly with coated rods until a uniform rough on the plate was observed, which were then placed into the incubator and incubated at 37℃ for 72 h. After the incubation, under the sterile operating stage, the growing single colonies were picked under the sterile operating stage and then further cultured in the medium with the appropriate alcohol concentration.

2.4.3 Amplified culture

After the growing single colony under the sterile operating stage, the single colony was removed with the inoculum ring and transferred to 25 mL liquid culture liquid containing the corresponding alcohol concentration for 72 h, 37℃ and 160 r / min.

2.4.4 Detection of ethanol dehydrogenase activity

ADH enzyme activity will be measured in different alcohol concentrations using an ADH kit as described below.

Table 2.1

Composition of the reagent box:

Reagent name	specifications	Save conditions
extract	Fluquid 110 mL 1 bottle	2 – 8°C for preservation
Powder one	Powder 1 bottle	2 – 8°C for preservation
Reagent a	Fluquid 20 mL x 1 bottle	2 – 8°C for preservation
Reagent 2	Two powder aphrodites	2 – 8°C for preservation
Reagent 3	Fluid 3 mL x 1 bottle	2 – 8°C for preservation

(2) Solution preparation:

1) Extract: pour the powder into the extract before use, the solution is suspension oil, and shake well before use;

2) Reagent 2: Before use, take 1 reagent 2 and add 1 mL of distilled water, which can be stored in-20 °C parts for 4 weeks to avoid repeated freezing and thawing. One can be made 100T, in order to prolong the use of the kit, then give one more.

(3) Product description:

ADH is a key enzyme in the metabolism of short-chain alcohol in living organisms, which catalyzes the reversible conversion of ethanol and acetaldehyde, and plays an important role in many physiological processes. Mammalian ADH is mainly generated in the liver, and liver damage leads to the release of ADH into the serum. High and low serum ADH activity reflects whether the liver function is abnormal.

ADH catalyzes the reduction of acetaldehyde by NADH to generate ethanol and NAD ′, and NADHI has an absorption peak at 340nm, while NAD^+None: Determine the rate of absorbance decrease at 340nm to calculate the ADH activity.

$$\text{Acetaldehyde} + \text{NADH}(340\text{m}) \rightarrow \text{Ethanol} + \text{NAD}^+$$

(4) Experimental instruments and experimental supplies:

Ice, cold ache, microplate, 96-well microplate, adjustable pipete, ultrasonic breaker, distilled water

(5) Operation steps:

1) Cell extraction

Take 5 mL of *Acetobacter* solution, centrifuged at 4℃, 5000 rpm for 10 min, discard the supernatant, take precipitation, add 1 mL of extract, break the ice bath sonication for 3min (power 300 w, sonicated for 3s, 7s, total time 3 min): 16000 g, centrifuged at 4℃ for 20 min, put the supernatant on ice for testing.

2) Determination steps

① Preheat the microplate reader for 30 min, and adjust the wavelength to

340 nm;

② Reagent 1 Thermal insulation in 25℃ water bath for more than 30 min;

③ Blank tube: add 20 μ L of distilled water, 8 μ L reagent 2,152 μ L reagent 1 and 20 μ L reagent 3 in 96-well plate, determine the change of absorbent value at 340 nm, record 15s and 75s absorbance, respectively, recorded as A1 and A2 respectively, Δ A blank tube =A1-A2;

④ Measurement tube: add 20 μ L of supernatant, 8 L reagent 2,152 μ L reagent 1 and 20 μ L reagent 3 in 96-well plate, and the absorbance changes were measured at 340 nm. The absorbance values at 15s and 75s were recorded as A3 and A4 respectively. Δ A Measurement of Tube =A3-A4.

The specific determination procedure is shown in the following Figure 2.1 (Appendix A) diagram of the enzyme activity determination of ADH:

(6) Calculation formula of enzyme vitality

Activity unit definition: 1 μmolNADH per minute per ml sample in 25℃ is 1 enzyme active unit.

ADH (U / mL) = [(Δ A test tube- Δ A blank tube) ε dV total 10^6]÷V appearance ÷T

= 2.68 (Δ A test tube- Δ A blank tube)

ε: NADH, 6.22 x103 L/mol / cm; d: 96 well plate optical diameter, 0.6 cm; V reverse total: total volume of reaction system, 200 μ L=210^{-4}L； 10^6: Unit conversion unit, 1 mol=110^6μ mol; Cpr: protein concentration of supernatant, mg/mL; W: Sample mass, g; V sample: liquid accumulation in the reaction system, 2 μ L = 0.02 mL; V Sample: extraction liquid volume, 1 mL; T: reaction time, 1 min; cell number: ten thousand.

2.4.7 Mutagenesis breeding program

(1) Primary screen: Take strain D with high ADH activity, divide the cell suspension into different tubules through aseptic operation, irradiate with ultraviolet light for 10min, 15min, 20min, 25min and 30min, then shake well and

apply 0.2ml on solid medium prepared containing 2% alcohol, and grow for 24 h at 30℃.

(2) Rescreening: the growing strains were selected for line culture, 37℃ for 24 h. After incubation, the growing colonies were selected and cultured for 5 generations to obtain *Acetobacter* with stable trait expression.

(3) Enrichment: Put the resulting stable colonies under the sterile operation stage, dip a few colonies and then inoculate into a liquid culture bottle with 2% alcohol concentration, 37℃, 160 r/min, and culture on the shaker for 24 h.

(4) Detection of ethanol dehydrogenase activity: take the supernatant after the enrichment culture for ethanol dehydrogenase activity detection, the method is the same as above.

CHAPTER III
EXPERIMENTAL RESULTS AND DISCUSSION

3.1 Isolation and screening of *Acetobacter* sp

After enrichment culture, the strain A coating in sterile environment, respectively coated in different alcohol concentration of plate, and then the coated medium placed in 30℃ constant temperature incubator for 36 h, after observe A strain growth, after comparing the strain in the alcohol concentration of 2% on the medium, and in the alcohol concentration of more than 2% medium is no growth, Figure 3.2 (Appendix A) is A strain growth in 2% of the medium. Following Figure 3.2 (Appendix A). Growth of acetic acid bacteria in vinegar fermented grains sample A in 2% alcohol medium

Of strain B enrichment for 36h, then on the ultranet workbench coating operation, the enriched B strains in alcohol concentration of 0%, 1%, 2%, 3%, 4% and 5% of solid medium in 30℃ incubator for 36 h, after culture strain B on different media have different growth (as shown below), can see the strain in the alcohol concentration of 2%, scattered flora in the medium alcohol concentration higher than 2%, so we select the two concentrations of the next step, following Figure 3.3 (Appendix B), Growth of acetic acid bacteria in sample B.

After enrichment culture of C strain, we coated strain C on the super clean table, the same coating on the solid medium of 0%, 1%, 2%, 3%, 4% and 5% of alcohol, then placed in a 30℃ incubator for 36h, after the cultivation of the strain D plate at different alcohol concentration, after comparing C strain in the alcohol concentration of 2% culture plate is very vigorous, appear on the medium higher than 2% alcohol concentration of scattered flora, following Figure 3.4 (Appendix B) Growth of acetic acid bacteria in alcohol-containing medium.

For D strain we take the same culture way, comparing the growth of D in each medium, D strain under different alcohol concentration growth is uniform, in the case of 2% alcohol colony morphology is obvious, so we select the alcohol

concentration of 2% medium growth strain D for the next step of screening, following Figure 3.5 (Appendix B) Growth of acetic acid bacteria in alcohol-containing medium.

The samples of different vinegar fermented grains were picked and grown in different alcohol concentrations, and the colonies with typical colony characteristics were further plate crossed, and then the single colonies were selected, and the operations were repeated 5 times. Stable single colonies were picked for further enrichment culture and ADH enzyme activity was measured. Underlined culture growth is shown below, following Figure 3.6 (Appendix C). Single colony culture of acetic acid bacteria in samples of vinegar fermented grains.

3.3 Preliminary determination of ADH enzyme activity

We can get information from Figure 3.7 (Appendix C) which is about ADH enzyme activity of acetic acid bacteria in samples of different vinegar fermented grains. Single colony-enriched solutions grown from various conditions were identified as described in the ADH enzyme assay. With the sample with distilled water as a blank tube, the ethanol dehydrogenase activity was measured for single colony samples A, B, C and D in media with different alcohol concentrations. The results are shown in Figure 3.2 (Appendix A), and the highest ethanol dehydrogenase activity of sample D under the culture condition of 2% alcohol concentration was 4.28810^{-2}U。Although the C sample also grew in the 5% alcohol concentration, its activity was not too high, indicating that the relationship between ADH enzyme activity and alcohol tolerance does not necessarily show a positive correlation. We selected strain D for 16sr RNA identification of *Acetobacter*.

3.4 Results of the mutagenesis culture experiments

After the screening, Strain D was the most active strain of ethanol dehydrogenase in the medium of 2% alcohol, So the strain D was subjected to UV mutagenesis, The ment solutions of their strains were placed and irradiated under UV light for 10min, 15min, 20min, 25min and 30min, Then then were coating culture on an ultra-clean table, After the incubation, To compare the growth status of the strains in the respective media, The shorter the UV irradiation time, The better the growth of the strain, Suggesting that UV has some effects on the strains, Further determination of ethanol dehydrogenase activity, The results are shown in Figure 3.8 (Appendix D). Found that the highest ethanol dehydrogenase activity after 30 min of irradiation. For the 8.25410^{-2}U, the mutagenized strain activity doubled over the original activity. And we can refer to Figure 3.8 (Appendix D) The ADH enzyme activity of acetic acid bacteria after mutagenic treatment.

CONCLUSIONS

According to the experimental results, the ADH enzyme activity of the cultured strains in sample D in 2% alcohol environment was 4.28810^{-2}U, further identified as *Acetobacter*, followed by UV mutagenesis of the most active strain D, after culture screening for ethanol dehydrogenase activity up to 8.25410^{-2}U variant strain D, which was obtained after 30 min of UV irradiation, has twice the higher activity than before mutagenesis.

The experimental results show that we can through artificial method, through the control variables of high activity ethanol dehydrogenase acetate screening, we can also through physical or chemical method of ADH activity artificial intervention, let it produce higher activity strains, the success of the experiment for high activity ethanol dehydrogenase research has a very important role.

Due to the time limit and the limitations of some scientific research conditions, there are still some deficiencies in this paper:

(1) The time of UV-mutagenesis can be further optimized;

(2) Conduct strain mutagenesis by plasma mutagenesis;

(2) The culture medium of ADH-producing *Acetobacter* can be further optimized to further improve the efficiency and stability of enzyme production.

REFERENCE

1. Jiang Xiaoying. Screening, identification and enzymatic properties of ethanol dehydrogenase producing strains [D]. Jimei University, 2013

2. Zhang Yunru. Breeding and kinetic characteristics of ethanol dehydrogenase strains [D]. Chongqing University, 2004

3. Zhao Li, Han Wei, Wang Jing, Chen Jiafeng. Chronic alcoholism and cerebrovascular disease [J]. Journal of Stroke and Neurology, 2005 (05): 477-478

4. Y.J. Yoo,J.S. Cho,S.H. Park,Y.R. Noh. Enzyme-based electrochemical denitrification[J]. Journal of Biotechnology,2010,150(S).

5. Chen Zhejiang. Breeding of Highly Active Acetobacillus and Extraction of Ethanol Dehydrogenase [D]. Shanghai Normal University, 2008

6. Lu Jian, Wei Yutuo, Huang Kun, Lu Chunhua, Huang Ribo. Construction of genetically engineered bacteria for producing fuel alcohol using glucose and xylose [J]. Guangxi Agricultural Biological Sciences, 2005 (03): 187-190

7. Wang Tao, Li Wen, Chen Yaqing. Optimization of Fermentation Medium for Ethanol Dehydrogenase Production by *Acetobacter* Acetate [J]. Grain, Oil and Food Technology, 2014,22 (01): 104-108. DOI: 10.16210/j. cnki-1007-7561.2014-01.011

8. Zeng Fancai, Li Hong, Shao Jun, Wu Minlu. Cloning and identification of the ADH1C * 1 allele of human ethanol dehydrogenase [J]. Journal of Luzhou Medical College, 2006 (01): 11-14

9. Wang Tao, Li Wen, Chen Yaqing. Optimization of Fermentation Medium for Ethanol Dehydrogenase Production by *Acetobacter* Acetate [J]. Grain, Oil and Food Technology, 2014,22 (01): 104-108. DOI: 10.16210/j. cnki-1007-7561.2014-01.011

10. Sun Weining. Research on bacterial flora in fermented grains of Luzhou liquor and pickled Chinese cabbage based on high-throughput sequencing [D]. Tianjin University, 2017

11. Li Yixin. Effect of crushing methods on ADH activity in acetic acid bacteria [J]. Food and Machinery, 2011,27 (05): 87-89

12. Chen Xiuyi, Chu Xufeng, Duan Weicheng, Xu Man, Wang Yixin, Pan An, Hu Ping. The effect of alcohol consumption on the gut microbiota of healthy adult males [J]. Chinese Journal of Microbiology, 2021,33 (09): 1009-1015+1020

13. Cao Guoliang, Qiao Yongfang. A case of cefoperazone sodium disulfide like reaction induced by rice wine [J]. Journal of Practical Diagnosis and Treatment, 2003 (04): 258

14. Chen Renju, Guo Dandan, Xiao Kaijun. Effect of pulsed strong magnetic field on enzyme activity in milk [J]. Food and Fermentation Industry, 2010,36 (11): 78-83. DOI: 10.13995/j.cnki.11-1802/ts.2010

15. Wu Hui, Li Lili, Li Xiaofeng, Yu Yigang. Study on in vitro sobering activity of corn peptide prepared by hydrolysis of immobilized enzyme in non-aqueous phase [J]. Food and Fermentation Industry, 2010,36 (12): 37-41

16. Zhang Xiaoxia, Mao Jianian, Zhang Yunli. Research Status of Ethanol Dehydrogenase Application [J]. Progress in Animal Medicine, 2007 (12): 92-95. DOI: 10.16437/j.cnki.1007-5038.2007.12.001

17. Yin Mingyu, Chen Min, Zhao Yimeng. Research progress on the metabolic characteristics of Acetobacillus and the mechanism of its anti alcoholic and liver protective effects [J]. China Brewing, 2021,40 (12): 6-11

18. ElAskri Taoufik,Yatim Meriem,Sehli Youness,Rahou Abdelilah,Belhaj Abdelhaq,Castro Remedios,DuránGuerrero Enrique,Hafidi Majida,Zouhair Rachid. Screening and Characterization of New *Acetobacter* fabarum and *Acetobacter* pasteurianus Strains with High Ethanol–Thermo Tolerance and the Optimization of Acetic Acid Production[J]. Microorganisms,2022,10(9).

19. Xin Lei, An Hui, Qin Guole, Luo Fengfeng, Li Xiuling. Ultraviolet mutagenesis and fermentation formula optimization of Bacillus subtilis XL05 strain [J]. Fermentation Technology Communication, 2020,49 (03): 142-146. DOI: 10.16774/j.cnki. issn.1674-2214.2020.03.004

20. Tang Tiantian. Gene expression and activity analysis of human ethanol dehydrogenase ADH1B2 [D]. Zhejiang University, 2011

21. Chen Xiuyi, Chu Xufeng, Duan Weicheng, Xu Man, Wang Yixin, Pan An, Hu Ping. The effect of alcohol consumption on the gut microbiota of healthy adult males [J]. Chinese Journal of Microbiology, 2021,33 (09): 1009-1015+1020. DOI: 10.13381/j.cnki.cjm.202109004

22. Zhou Bingchen. Study on the relationship between the activity of acetic acid bacteria ethanol dehydrogenase and acid production rate in vinegar production [J]. China Brewing, 2009 (11): 58-59

23. Liu Yuetao,Cai Congcong,Qin Xuemei. Regulation of gut microbiota

24. Su Zhiping. Mechanism of gut microbiota enhancing the immune activity of Drosophila melanogaster system [D]. Fujian A&F University, 2022. DOI: 10.27018/d. cnki. gfjnu.2022.000490

25. Peng Mei, Yang Juan, Li Liangqun, Yang Xiaosheng, Yu Huaying, Sun Bin, Luo Zhongsheng, Yang Yan, Pan Xiong, Cai Yong. Studies on the Chemical Constituents and Antibacterial Activities of High Efficiency Liquor Yeast and Acetobacillus Fermented Coral Ginger before and after Fermentation [J]. China Brewing, 2022,41 (02): 80-85

26. Shi Huiqin, Zhang Gong, Zhang Yanmin, Chi Huanrong, Li Pei, Li Zhaofei, Zhao Guozhong. Isolation of Acetobacillus from Dahong Zhejiang Vinegar and Analysis of Key Amino Acids for Acid Production [J]. China Brewing, 2022,41 (02): 34-39

27. Yin Mingyu, Chen Min, Zhao Yimeng. Research progress on the metabolic characteristics of Acetobacillus and the mechanism of its anti alcoholic and liver protective effects [J]. China Brewing, 2021,40 (12): 6-11

28. Wang Lu, Wei Bofan, Li Miaomiao, Li Xiaozhe, Wang Bo, Kan Yunchao, Qiao Huili. Effects of Lactobacillus plantarum, *Acetobacter* mali and Saccharomyces cerevisiae on the behavior and development of Drosophila melanogaster [J]. Acta Entomologica Sinica, 2021,64 (04): 460-470. DOI: 10.16380/j.kcxb.2021.04.005

29. Wan Ningwei, Lei Bangxing, Li Biao, Yang Yue, He Jin. Study on microbial diversity and dynamic changes of organic acids during the fermentation process of Baisuan Tang [J]. Food and Fermentation Industry, 2022,48 (09): 65-70. DOI: 10.13995/j.cnki.11-1802/ts.028592

30. Zhu Huixia, Sun Jinxu, Gao Yue, Wang Qian, Li Xuezhen. Study on the bacterial changes during fruit enzyme fermentation process [J]. Shandong Chemical Industry, 2020,49 (22): 20-26. DOI: 10.19319/j.cnki. issn.1008-021x.2020.22.008

31. Gao Ling, Wu Xiaodan, Jin Zhengyu, et al. Improving *Acetobacter* pasteureus acetic acid production efficiency by regulation of ethanol oxidation pathway enzymes and cofactor metabolism [C]//China food science and Technology Society and the summary of the 17th annual meeting of China food science and Technology Society [Unknown publisher], 2020:296-297. DOI: 10.26914/c.cnkihy.2020.021950

32. Zhao Hui. Isolation, Identification, Infection Mechanism, and Biological Control of the Pathogen of Pleurotus ostreatus Yellow Spot Disease [D]. Shanxi Agricultural University, 2019. DOI: 10.27285/d. cnki. gsxnu.2019.000324

33. Yin Xuwen, Xing Junde, Sun Zhiwei. Study on the oxidation of alcohol to acid by *Acetobacter* sp. Shanghai 1.01 [J]. chemical engineering, 2018,46 (07): 1-6

34. Li Yujuan. Study on the Promotion of Drosophila Growth and Development by Acetobacillus Orientalis through Insulin Signal [D]. Shanxi Medical University, 2017

35. Wang Jing. Knockout of the uvrA gene of *Acetobacter* pasteuricus and its impact on bacterial acetic acid tolerance [D]. Tianjin University of Science and Technology, 2016

36. Zhu Chuanliu, Xia Xiaole, Shi Wei, Wang Wu. The effect of acetic acid stress on the activity of acid-producing respiratory chain in *Acetobacter* pasteurianus [J]. Microbiology Bulletin, 2017,44 (10): 2269-2279. DOI: 10.13344/j. microbiol.china.160861

37. Wang Jing. Knockout of the uvrA gene of *Acetobacter* pasteuricus and its impact on bacterial acetic acid tolerance [D]. Tianjin University of Science and Technology, 2016

38. Ma Xinfeng, Chen Yilun, Zhou Bo, Li Chaonan, Wu Hui, Huang Yingqian, Zhang Yuhuan, Guo Shasha. Physiological response of Acetobacillus pasteuricus Hu Niang 1.01 to acetic acid stress in liquid storage [J]. Food and Fermentation Industry, 2016,42 (01): 42-47. DOI: 10.13995/j.cnki.11-1802/ts.201601008

39. Zhu Yunping, Li Dawei, Zhang Xue, Li Xiuting, Liang Zhijun, Sun Baoguo, Yang Ran. Optimization of Culture Conditions and Medium Composition for *Acetobacter* Pasteuri B103 [J]. China Food Additives, 2015 (06): 60-65

40. Zhu Yunping, Li Dawei, Zhang Xue, Huang Yueyi, Li Xiuting, Liang Zhijun, Sun Baoguo. Optimization of Fermentation Conditions for Tropical *Acetobacter* B104 [J]. China Brewing, 2015,34 (05): 30-34

41. Zhang Wen, Study on the Effect of Ion Beam Irradiation on *Acetobacter* Production of Bacterial Cellulose (BC) Shaanxi Province, Shaanxi University of Science and Technology, 2013-12-01

42. Zhang Wen, Zhao Qiuhong, Li Yanjun. Research on *Acetobacter* Fermentation of Bacterial Cellulose and Its Modification [J]. Food Industry Technology, 2013,34 (17): 181-184. DOI: 10.13386/j.issn1002-0306.2013.17.081

43. Shen Qiuhua, Bai Wenjie. Optimization of *Acetobacter* Pasteuri As1.41 Culture Medium [J]. Anhui Agricultural Science, 2012,40 (36): 17455-17456+17459. DOI: 10.13989/j.cnki.0517-6611.2012.36.046

44. Zhang Keping, Zheng Yu, Jia Junhui, Luo Jianmei, Wang Min. Cloning and protein sequence analysis of alcohol dehydrogenase gene from *Acetobacter* Pasteuri AC2005 [J]. Food Industry Technology, 2011,32 (12): 265-267+420. DOI: 10.13386/j.issn1002-0306.2011.12.001

45. Zhou Zhu, Zha Fan, Zhang Qin, Chen Zhen, Chen Xiangyu, Hu Yong'an. Klebsiella sp. WL1316 Overexpression of alcohol dehydrogenase gene improves ethanol production efficiency [J]. chemical engineering, 202

46. Liu Jing. Directed Immobilization of Ethanol Dehydrogenase with Magnetic Nanoparticles and Application Research [D]. Nanchang University, 2021. DOI: 10.27232/d.cnki.gnchu.2021.002017

47. Zhao Tianyuan. The protective effect and mechanism of ethanol dehydrogenase inhibitors on liver fibrosis [D]. Zhengzhou University, 2020. DOI: 10.27466/d. cnki. gzzdu. 2020. 004575

48. Luo Shanshan. Clinical application value of alcohol dehydrogenase and aldehyde dehydrogenase gene family in gastric cancer and exploration of ALDH4A1 mechanism [D]. Guangxi Medical University, 2019

49. Lv Yunbin. Coexpression of ethanol dehydrogenase and acetaldehyde dehydrogenase in lactic acid bacteria and their protective effect on acute alcoholic liver injury in mice [D]. Nanjing Agricultural University, 2017. DOI: 10.27244/d.cnki. gnjnu.2017.000221

50. Wang Heng, Li Yongsheng, Gao Xiufeng, Tian Tian, Lin Yulian. A cyclic catalytic flow analysis method for determining ethanol dehydrogenase activity [J]. Brewery Technology, 2010 (10): 100-102+114. DOI: 10.13746/j.njkj.2010.10.004

51. Bao Jianjun, Bao Tianhao, Wu Wenyu, Zhang Xulan, Wu Xuemei, Wu Hao, Liu Hongyan. Study on the relationship between different genotypes of

alcohol dehydrogenase and cognitive impairment in alcohol dependent patients [J]. Chinese Journal of Drug Abuse Prevention and Control, 2010,16 (03): 136-140. DOI: 10.15900/j.cnki.zylf1995.2010.03.011

52. Wu Guiying, Jin Fang, Wu Yuanxin. Study on Fermentation Conditions for Ethanol Dehydrogenase Production by Saccharomyces cerevisiae [J]. Food Industry Science and Technology, 2009,30 (11): 176-177+190

53. Song Gang, Su Dongmei, Ge Jingping, Ling Hongzhi, Liu Songmei, Ping Wenxiang. Study on alcohol dehydrogenase activity of beer yeast HD34-1 [J]. food science, 2008,29 (11): 439-441

54. Qin Lina. Overexpression of Ethanol Dehydrogenase I Gene in Saccharomyces cerevisiae [D]. Fujian Normal University, 2008

55. Zhao Anqi. Cloning and expression analysis of ADH gene of Hemerocallis fulva 'Qiuhong' under seawater stress [D]. Shanghai University of Applied Technology, 2022. DOI: 10.27801/d. cnki. gshyy.2022.000223

56. Ma Zhiwei, Teng Jiping, Zhuang Bufeng. The relationship between single nucleotide polymorphism of the alcohol dehydrogenase 1B-rs1229984 gene and clinical characteristics and prognosis of esophageal cancer patients [J]. Chinese Journal of Practical Diagnosis and Treatment, 2019,33 (06): 557-561. DOI: 10.13507/j.issn.1674-3474.2019.06.011

57. Cai Yao, Gong Shuangyan, Chen Yingqi, Li Youyou, Li Xiaojing, Xu Zhiwen, Zhu Ling. Research progress on the mechanism of bifunctional aldehyde/ethanol dehydrogenase AdhE participating in bacterial host interaction [J]. Microbiology Bulletin, 2019,46 (06): 1530-1536. DOI: 10.13344/j.microbiol. china.180608

58. Li Jing. The relationship between the activities of ethanol dehydrogenase and acetaldehyde dehydrogenase and the occurrence of liver cancer [D]. Zhengzhou University, 2017

59. Chen Yang. Research and Application of Characteristics of Ethanol Resistant Acetic Acid Bacteria [D]. Hubei University of Technology, 2016

60. Huang Fei, Xi Chaoqun, Huang Yanhua, Chen Jianhua, Hu Lei. Cloning, expression, and activity optimization of ethanol dehydrogenase [J]. Chinese Medical Biotechnology, 2015,10 (04): 361-365

61. Huang Fei, Xi Chaoqun, Huang Yanhua, Chen Jianhua, Hu Lei. Cloning, expression, and activity optimization of ethanol dehydrogenase [J]. Chinese Medical Biotechnology, 2015,10 (04): 361-365

62. Huang Fei, Xi Chaoqun, Huang Yanhua, Chen Jianhua, Hu Lei. Cloning, expression, and activity optimization of ethanol dehydrogenase [J]. Chinese Medical Biotechnology, 2015,10 (04): 361-365

63. Liang Yan. Effects of non flooded and flooded environments on the expression of ethanol dehydrogenase 3 in young roots of rice and upland rice [J]. Jiangsu Agricultural Science, 2013,41 (09): 22-23. DOI: 10.15889/j.issn.1002-1302.2013.09.92

64. Tian Hongbo, Chen Yuguo, Xu Feng, Zhang He, Zhang Yun. Association between alcohol dehydrogenase 2 gene polymorphism and type 2 diabetes in Shandong Han patients with coronary heart disease [J]. Chinese Journal of Gerontology, 2013,33 (01): 67-68

65. Liu Cunde, Shen Quanguang, Zhang Guozheng, Yan Longfei, Tang Peisong. Changes of alcohol dehydrogenase and induction of its isoenzymes during wheat seed germination [J]. Journal of Plant Physiology, 1980 (04): 361-367

66. Li Tao, Lu Gaihui, Zhao Min. Meta analysis of association between gene polymorphisms of circadian protein, alcohol dehydrogenase 4, hypothalamic secretin receptor 2 and cluster headache [J]. Journal of Clinical neurology, 2022,35 (01): 8-15

67. Liu Lijun. Study on epigenetic modification changes and ADH1 function in alpine ion mustard under cold stress [D]. Lanzhou University, 2017

68. Song Yanjun. Study on the correlation between ADH1 gene and drug resistance and pathogenicity of Candida albicans [D]. Jinan University, 2017.

Appendix A

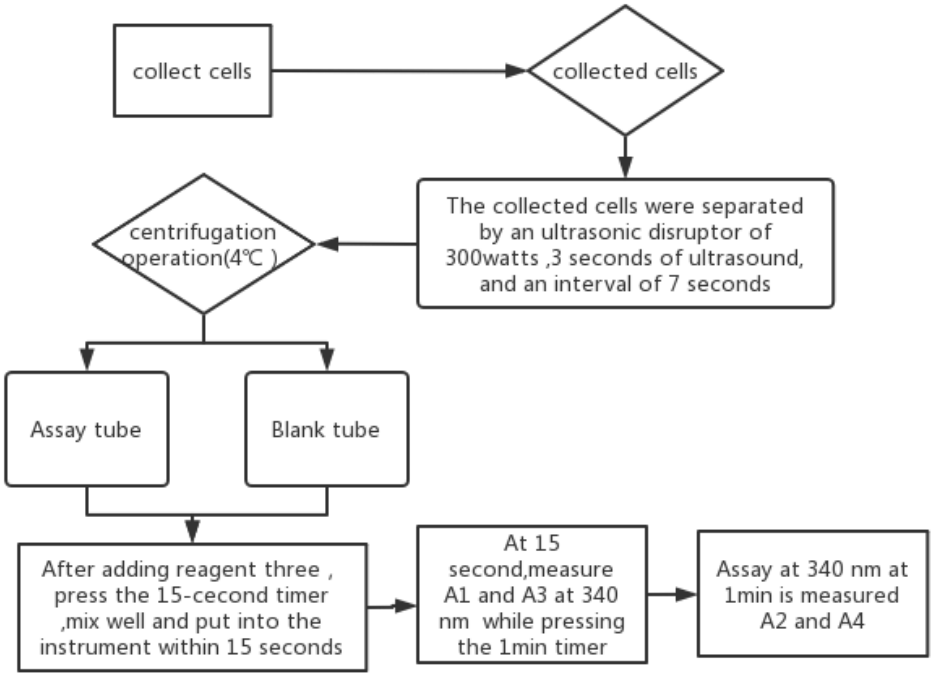

Figure 2.1. Enzyme activity determination of ADH

Figure 3.2. Growth of acetic acid bacteria

Appendix B

Figure 3.3. Growth of acetic acid bacteria in sample B

Figure 3.4. Growth of acetic acid bacteria in alcohol-containing medium

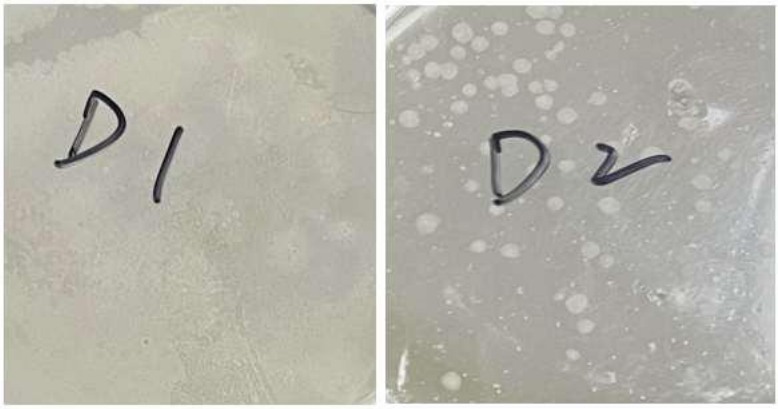

Figure 3.5. Growth of acetic acid bacteria in alcohol-containing medium

Appendix C

Figure 3.6. Single colony culture of acetic acid bacteria in samples of vinegar fermented grains

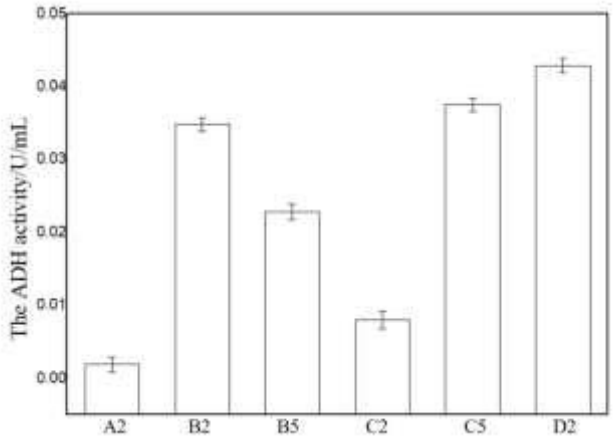

Figure 3.7. ADH enzyme activity of acetic acid bacteria in samples of different vinegar fermented grains

Appendix D

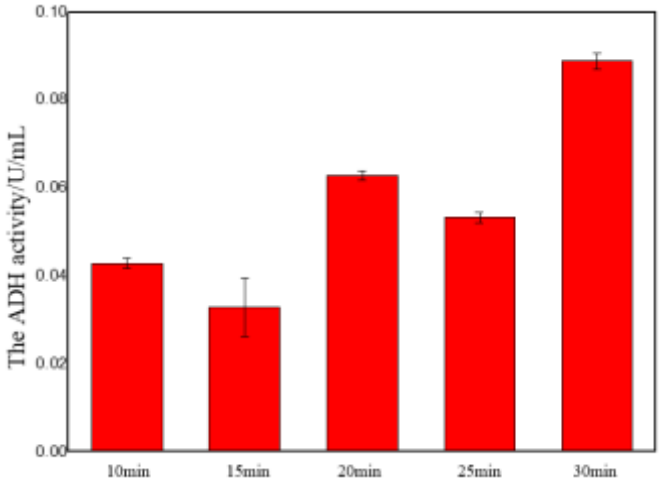

Figure 3.8. The ADH enzyme activity of acetic acid bacteria after mutagenic treatment