Three-Factor Joint Shock Breeding Yeast

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Abstract: through three-factor (high gravity ethanol, high gravity sodium chloride and heat) joint shock test, we found that different yeast species have different sensitivity to temperature. For example, the mutants resulting from 45℃ and 50℃shock of rice wine yeast can ferment in medium containing 17% ethanol, and they have similar ethanol production ability; but the mutant resulting from 50℃ shock of brewer's yeast can produce more ethanol than mutant resulting from 45℃ shock. Through 48 hours fermentation, original rice yeast can produce 2.6% alcohol in mash, while some of the mutants from this yeast can produce 3.1% alcohol; original brewer's yeast can produce 2.9% alcohol in mash, while some of the mutants of brewer's yeast can produce 3.8% alcohol. So the way of three-factor joint shock can improve yeast ability to produce ethanol and ethanol tolerant capacity significantly.

Keywords: shock, yeast, ethanol, fermentation

1 Introduction

VHG[1] (very high gravity)fermentation is a hot point of fuel ethanol research, because VHG fermentation can reduce production cost of ethanol and energy consumption evidently.VHG fermentation need to solve the problem of suppression resulting from high gravity ethanol, hyperosmosis and high temperature. What is the suppression mechanism? Study shows that the accumulation of ethanol in yeast cultures leads to decreases in yeast growth, and ethanol formation[2]. Ethanol seems to diffuse through the yeast cell plasma membrane without involvement of carriers and does not accumulate in opposition to a concentration gradient of external alcohol in the cultures of *Saccharomyces cerevisiae*[3]. The interference of ethanol with cell membrane structure and transport has been suggested as the primary cause for suppression [4].

How does yeast overcome enviroment stress (including high gravity alcohol, hyperosmosis and so on)? Study shows that yeast cells grown in the presence of ethanol showed changes in the lipid composition (higher contents of both ergosterol and unsaturated fatty acid) of the cell plasma membrane and tolerance to high levels of external ethanol improved [5,6]. Trehalose[7,8] is well known as a cell protector against environmental stress and high cellular levels of this sugar were observed when the fermentation ceased [9].

One way of solving the problem above is obtaining excellent yeast. How to obtain high quality yeast? There are many ways. Shock is a good way to obain yeast, shock includes heat, high gravity salt[10] ,high gravity ethanol and so on.Actually, shock and ultraviolet raying are used jointly in breeding yeast. There is no report about multi-factory shock breeding yeast, so we have studed the way of three-factor(high gravity ethanol , high gravity salt and heat) joint shock breeding yeast in this paper.

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2Experiments

2.1 The main experimental apparatus and materials

Electric constant temperature water bath (DZKW-4), Electric Ovens (Z02-0), temperature controller incubator (DHP-781), Refrigerator (BCD-182A), electronic balance (Mettler AE240), centrifuge (T800), spectrophotometer (PE), Autoclave (Autester E 30DRY), pallet scale(IPT-10), sterile console, alcohol meter(MC), test tubes, flasks, a variety of agents.

2.2 Medium

Medium 1: cassava mash (containing 20% total sugar, 15% reducing sugar, 5 ‰ urea, 2 ‰ potassium dihydrogen phosphate), pH 4.5.

Medium 2: medium 1 containing 17% ethanol and 5% sodium chloride.

Maltose medium 1(containing 13% maltose, 2% agar, 2 ‰ urea, 2‰ potassium dihydrogen phosphate, 2 ‰ magnesium sulfate), pH 4.5.

Maltose medium 2(containing 13% maltose, 2% agar, 2 ‰ urea, 2 ‰ potassium dihydrogen phosphate, 2 ‰ of magnesium sulfate, 17% ethanol), pH 4.5.

Original Yeast: brewer's yeast (*Saccharomyces cerevisiae*), rice wine yeast (*Saccharomyces cerevisiae*)

Test method: DNS method is used to determine the content of total sugar and reducing sugar, distillation is used to determine alcohol content of mash.

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2.3 Experiment Design

2.3.1 Experiment of yeast ethanol tolerance capacity

First step: making up medium containing 10%, 11%, 12%, 13% and 14% alcohol respectively; then taking 15ml medium above to sterilized test tubes respectively.

Second step: taking 1ml activated yeast into the medium above, respectively; then culturing them for 3 days and observing the growth of yeast.

2.3.2 Experiment of three-factor joint shock

First step: activating yeast in 30℃ water bath;

Second step: adding the activated yeast to the medium 2,mixing evenly, and immediately placing them in 40℃, 45℃, 50℃ and 55℃ water bath in 10min respectively, then placing them in 30℃ water bath for 5 days and observing the growth of yeast.

2.3.3 Experiment of mutants isolating

Mutants grown well are chosen, and seeded to maltose medium 2, and incubated 3 days. The strains growing well in maltose medium 2 the strains growing well in maltose medium 2 are chosen again, seeded to new maltose medium 2, cultured for 3 days, the strains growing will are pure strains. Finally, the pure strains are saved.

2.3.4 Test of yeast fermentation producing ethanol

The way of yeast inoculation: 10-fold amplification; Mash: medium 1;

Fermentation parameters: the fermentation time is 48 hours, fermentation temperature is 32℃;

Determination parameters: residual total sugar and residual reducing sugar, concentration of alcohol of mash, weight of fermentation broth (Weighing the weight of the fermentation broth Every 6 hours or 12 hours).

3 Results and analysis

3.1 ethanol tolerance capacity test of the rice wine yeast and brewer's yeast

Table 1 test result of yeast ethanol-resistant ability

Yeast	Ethanol concentration				
	10%	11%	12%	13%	14%
A	√	√	√	√	×
B	√	√	×	×	×
C	√	√	√	√	×

(Note: A is Brewer's yeast .B is Rice wine yeast. C is Angel super dry yeast; growth of yeast is judged with the bubbles production.)

As shown as table 1, brewer's yeast can ferment in medium containing 13% ethanol at most, rice wine yeast can ferment in medium containing 10% ethanol at most, Angel super dry yeast can ferment in medium containing 12% ethanol at most.

3.2 Results and analysis of three-factor joint chock test

As shown as table 2, 20% of mutants resulting from 40℃ heat shock of rice wine yeast can ferment normally, 60% of mutants resulting from 45℃ heat shock of this strain can ferment normally, 20% of mutants strain resulting from 50℃ heat shock of this strain can ferment normally, all of this yeast die with 55℃ heat shock. all of brewer's yeast die with 40℃ heat shock, 60% of mutants resulting from 45℃ heat shock of this strain can ferment normally,20% of mutants resulting from 50℃ heat shock of this strain can ferment normally, all of this yeast die with 55℃ heat shock.

Table 2. The survival rate of mutant

Yeast	40℃	45℃	50℃	55℃
A1	×	√	×	×
A2	×	√	×	×
A3	√	√	×	×
A4	×	×	√	√
A5	×	×	×	×
B1	×	×	√	×
B2	×	√	√	×
B3	×	√	×	×
B4	×	√	×	×
B5	×	×	√	×

(Note: the fermentation broth is observed in 120 hours. if any broth produces bubbles continuously more than 3 days, mutant in this broth seems to survive, said with a √; otherwise, the mutant seems as dead, said with ×. A kind of yeast needs to do five parallel experiments in each temperature conditions. A stands for rice wine yeast, B stands for brewer's yeast.)

3.3Ethanol production experiment of yeast

3.3.1 Test of ethanol production capacity of rice wine yeast and brewer's yeast

As shown as table 3, Angel yeast's ethanol production ability is best, the rice wine yeast's is worst.

Table 3. Ethanol production capacity experiment of original yeast

Strain	The ethanol concentration of Mash (%)	Residual total sugar content (%)	Residual reducing sugar content (%)
A	2.6	4.2	1.9
B	2.9	4.0	1.8
C	3.6	2.8	0.9

(Note: A is rice wine yeast, B is brewer's yeast, C is Angel super dry yeast.)

3.3.2 Test result of mutant strains ethanol production capacity

Table 4. Mutants' ethanol production capacity

strains	Ethanol concentration in Mash (%)	Residual total sugar content (%)	Residual reducing sugar content (%)
A3(40℃)	2.7	3.4	1.4
A1(45℃)	2.6	3.5	1.3
A2(45℃)	3.1	3.4	1.4
A3(45℃)	2.8	3.1	1.0
B2(45℃)	0.2	6.3	2.8
B3(45℃)	0	5.2	3.3
B4(45℃)	0.2	2.9	0.5
A4(50℃)	2.6	2.3	0.3
B1(50℃)	1.8	2.7	0.4
B2(50℃)	3.5	3.7	0.8
B5(50℃)	3.8	2.6	0.8

As shown as table 4, mutants resulting from40℃, 45℃ and 50℃ heat shock of rice wine yeast have better ability to produce ethanol than original yeast, so the temperature of 40℃, 45℃ and 50℃ is good for heat shock of rice wine yeast. Mutants resulting from 50℃ heat shock of brewer's yeast have better ability to produce ethanol than original yeast, so the temperature 50℃ is good for heat shock of brewer's yeast. in short, different yeasts need different shock temperature.

Through this experiment, it seems that the strains A2 (45℃), A4 (50℃), B2 (50℃), and B5 (50 ℃) are development potential strains.

4 conclusion sand recommendations

4.1 Conclusion

⑴ Trough three-factor (high gravity ethanol, high gravity sodium chloride and heat)joint shock, yeast's ethanol tolerance capacity can be significantly increased. original rice wine yeast can grow in medium containing 11% ethanol, but its mutant strains can ferment in medium containing 17% ethanol; original brewer's yeast can grow in medium containing 13% ethanol, but its mutant strains can grow in medium containing 17% ethanol; the mutant strains of brewer's yeast and rice wine yeast have better ethanol tolerance capacity than angel super dry yeast.

⑵Using the medium containing 20% total sugar and 15% reducing sugar, mutants resulting from 40℃, 45℃ and 50℃ heat shock of rice wine yeast have better ability to produce ethanol than original yeast, but mutants resulting from 50℃ heat shock of brewer's yeast have better ability to produce ethanol than original yeast; ethanol production ability of the mutants is similar to angel super dry yeast.

⑶Mutants have high tolerant ethanol capacity, while ethanol production ability of them may be not good.

4.2 recommendations

⑴In future test, the optimal shock conditions (the concentration of ethanol and sodium chloride, temperature, shock time) need to be determined;

⑵The genetic stability of mutants which have good ethanol production ability need to be determined, and practicable strains need to be isolated finally;

⑶The optimal fermentation conditions need to be determined, including fermentation temperature, oxygen, stirring, pH and adding nutrients.

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