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Full Length Research Paper

Screening of higher yield of ethyl acetate yeast and researching of ester-producing conditions

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A higher yield of ethyl acetate yeast Y1 was selected from Daqu and Xiaoqu, and the production volume of ethyl acetate was 2.152 g/l when cultured in corn saccharification mash. The strain is identified as *Hansenula anomala*. Then, through the single factor test to study the effects of diffident culture mediums and diffident culture methods on ester-producing, the results showed that the best ester-producing culture medium for Y1 yeast was the wheat saccharification mash. The ester yield of static culture was 40% higher when compared with shaking culture. several factors were selected to carry on the orthogonal test once more, the result indicated that the optimization plan were as follows: brix was 8°, pH was 5, temperature was 25°C, culture time was 4 days, and ethyl alcohol was 2%, total ester production of Y1 was 4.812 g/l in this condition.

Key words: Ester–production yeast, culture method, ester production condition, orthogonal test.

INTRODUCTION

Ester–producing yeast which has strong oxidation characteristics and ester producing capability is one of the main flavor species in liquor (Wu, 1994). Ester plays a very important role in liquor fragrance; its content is more than other aroma components that is one of the major features different from other distilled spirits. Lactic acid ethyl ester, ethyl acetate and ethyl caproate account for 90% of total ester in liquor (Shen, 1980). Therefore, the study of liquor flavor quality must focus on esters, especially the ethyl acetate, which synthesis in the cell of the ester-producing yeast, rather than compound by the esterification in the medium (Wang and Zhao, 2003).

This study mainly focus on screening high ethyl acetate producing yeast, then through single factor test and orthogonal test to explore the optimum conditions of ester-producing. It has significant economic benefits on the application of the ester- producing yeast. It also has positive significance for reforming traditional liquor production techniques.

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MATERIALS AND METHODS

Yeast strain is isolated from *Daqu* and *Xiaoqu*. Saccharifying enzyme, wheat, barley, corn, sorghum, and rice is brought from the market. YEPD liquid medium includes: glucose (2%), yeast extract (1%), and peptone (2%). Phenolphthalein indicator, hydrochloric acid standard titrate solution and sodium hydroxide standard titrate solution were also used.

The preparation of corn saccharification mash

The preparation of corn saccharification involves, smashing the corn by grinder and adding some water, then gelatinate by heating for 1 h. Cool to 60°C and add some saccharifying enzyme, saccharify for one hour at 60°C then filter and adjust the brix to 8°.

Screening of high yield of ethyl acetate yeast

17 yeasts were isolated from *Daqu* and *Xiaoqu* and numbered as Y1 to Y17, after activated culture, inoculated into the corn saccharification mash medium (100 ml culture medium per 300 ml flask). Inoculation amount was 0.2 ml, static culture four days at 30°C. Then, 80 ml ethanol was added into the broth and 100ml distilled fluid of the broth was collected and the content of ethyl acetate of the broth on gas chromatography was determined.

Analysis of the product components of the yeast

Take some broth of the yeast (the highest yield of ethyl acetate yeast selected from the yeasts) and centrifuge at a speed of 10000 r/min, then, analyze the product on gas chromatography (GC). At the same time, prepare the solution of eight kinds of standard ester sample, analyze it on GC under the same chromatographic conditions.

Identification of the yeast

Identify the yeast (the highest yield of ethyl acetate yeast) by VITEK －32 automatic microorganism identification instrument (Zhang et al., 1999; Wang et al., 2004).

Effect of different mediums on ester yield

According to the method of the preparation of corn mash, wheat, corn, barley, sorghum, and rice were used to prepare the saccharification mash medium respectively. Activate the yeast and inoculate into the saccharification mash mediums respectively. Inoculation amount was 0.2 ml. static culture was four days at 30°C. Then, 80 ml ethanol was added into the broth and 100 ml distilled fluid was collected from the broth, and this determined the content of total ester.

Effect of different culture methods on ester yield

Four kinds of culture methods were designed: static culture, 50 r/min shaking culture, 100 r/min shaking culture and 150 r/min shaking culture. The saccharification mash was used to determine this test as the culture medium. Other operations were carried out according to the test of the determination of the optimum medium

Orthogonal test

Five factors were designed (brix, pH, alcohol content, temperature and time) in four levels of orthogonal test (Xu et al., 1999), and 0.1 N hydrochloric acid was used to adjust the pH, while 95% alcohol was used to adjust the ethanol content of the medium. Ethanol was added the after sterilization of medium.

The measurement of total ester was determined by Saponification reflux method (Yi, 2008; Guo, 2009) while the measurement of ethyl acetate was determined by gas chromatography (Cesar et al., 2009; Chen, 2009).

RESULTS

Screening of high yield of ethyl acetate yeast

The results of the screening of high yield of ethyl acetate yeast were given in Table 1. The production of ethyl acetate of the Y1 yeast was the highest. It was 2.152 g/l, far higher than other yeasts. So, the Y1 yeast was selected to research the optimum ester-producing conditions

Table 1. Screening of high yield of ethyl acetate yeast.

Strains	Ethyl acetate (g/l)
Y1	2.152
Y2	0.090
Y3	0.361
Y4	0.690
Y5	0.242
Y6	0.435
Y7	0.909
Y8	0.638
Y9	0.372
Y10	0.152
Y11	0.456
Y12	0.184
Y13	0.124
Y14	0.033
Y15	0.192
Y16	0.135
Y17	0.823

Analysis of the product components of the yeast

Because gas chromatograph has the function of qualitative analysis, it is feasible to use gas chromatograph to analyze the product components of the yeast. Figure 1 was the gas chromatogram of the eight kinds of standard ester sample, it showed the retention times of the eight standard esters, for example, the retention time of ethyl acetate was 1. 058 min, ethyl caproate was 4.715 min, ethyl lactate was 9.699 min. Figure 2 was the GC of the product of the yeast Y1; the Y1 yeast mainly produces ethyl ester in fermentation when compared with Figure 1.

Identification of the yeast

As Figure 3 showed, Y1 colonies are white, neat edge, drier, lipid surface. The microscope images (Figure 3) revealed that the reproduction of Y1 strains mainly was budding. Used the VITEK－32 automatic microorganism identification instrument to identify the Y1 yeast, the result indicated that Y1 yeast was *Hansenula anomala* (Figure 4)

Effect of different mediums on ester yield

The productions of total ester of the Y1 in different mediums were given in Table 2. The production of ester in wheat saccharification mash medium was the highest, average 3.501 g/l. Therefore, make sure that wheat saccharification mash is the medium to research other ester-producing conditions.

Effect of different culture methods on ester yield

The productions of total ester of different culture methods

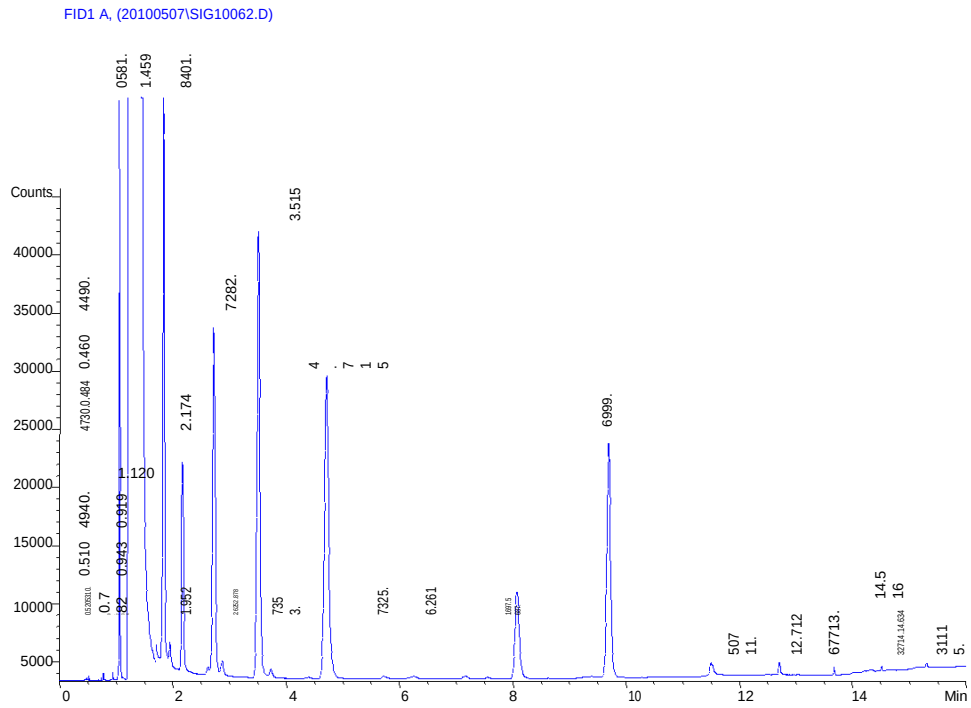


Figure 1. Chromatogram of eight standard esters.

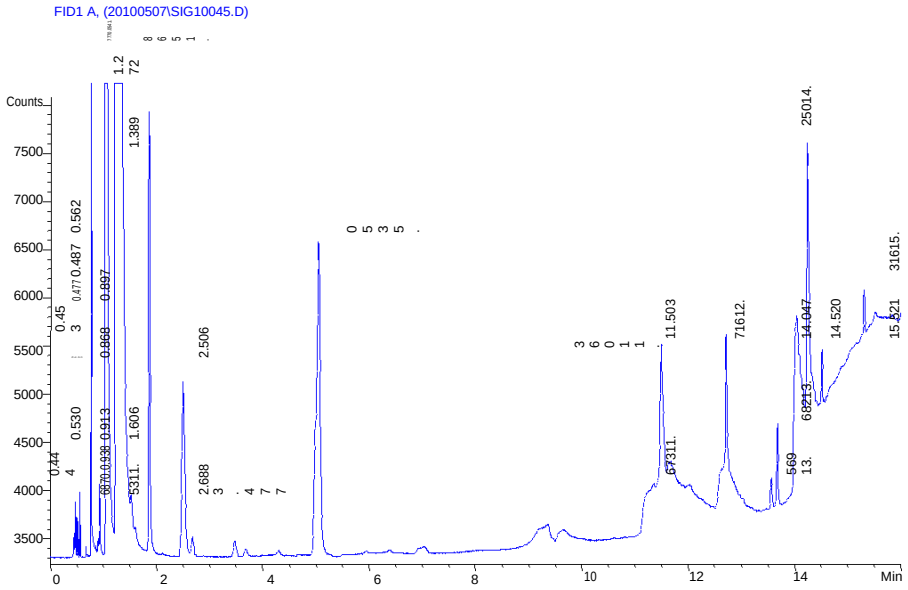


Figure 2. Chromatogram of the product of Y1.

were given in Table 3. Obviously, the production of total ester of static culture is higher than shaking culture, with an average of 3.320 g/l; although it is 40% higher than shaking culture. When culture is shaken, the production of total ester reduces gradually as the shake speed increase, it may be that more ventilation volume enhance the breath of the yeast and accelerate the decomposition of ester. So the best culture method for the yeast Y1 was static culture.

Orthogonal test

Through the orthogonal test to study the effect of brix, pH, ethanol content, temperature and time on the ester production of Y1 yeast, the result was given in Table 4,

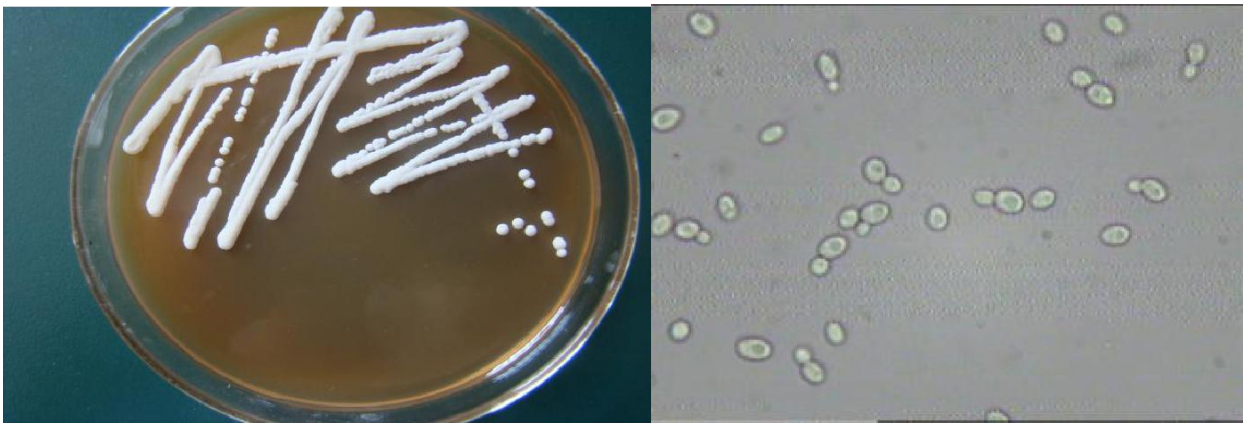
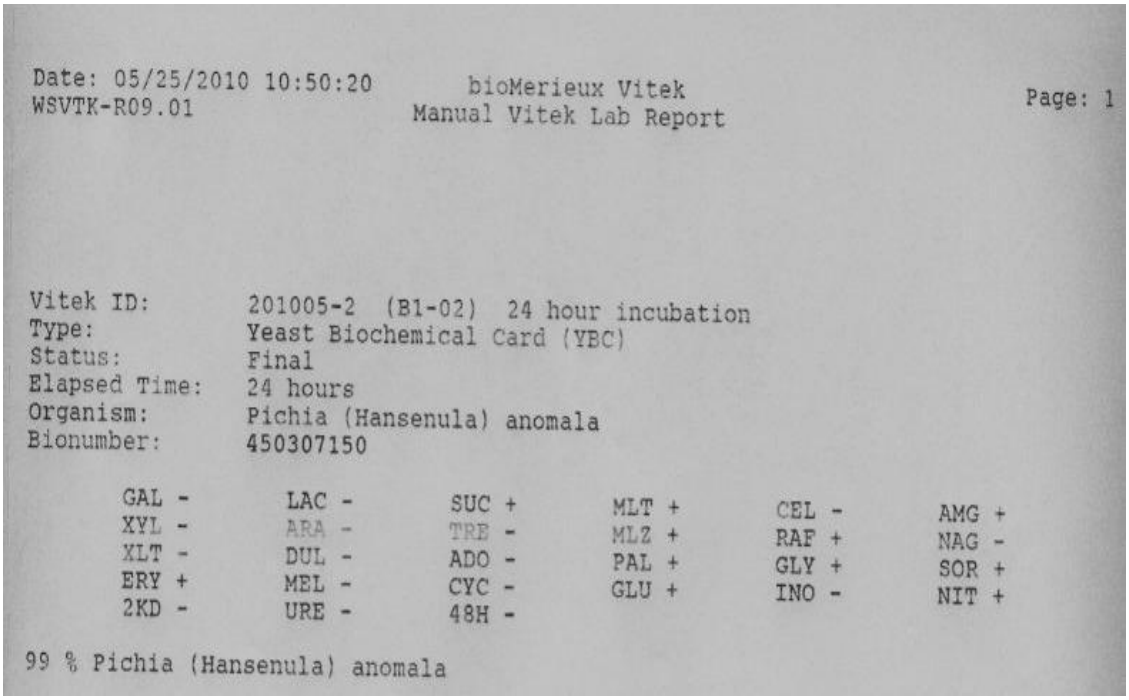


Figure 3. The colonies and cells picture of Y1 (400 ×).



Date: 05/25/2010 10:50:20 bioMerieux Vitek Page: 1
WSVTK-R09.01 Manual Vitek Lab Report

Vitek ID: 201005-2 (B1-02) 24 hour incubation
Type: Yeast Biochemical Card (YBC)
Status: Final
Elapsed Time: 24 hours
Organism: Pichia (Hansenula) anomala
Bionumber: 450307150

GAL -	LAC -	SUC +	MLT +	CEL -	AMG +
XYL -	ARA -	TRE -	MLZ +	RAF +	NAG -
XLT -	DUL -	ADO -	PAL +	GLY +	SOR +
ERY +	MEL -	CYC -	GLU +	INO -	NIT +
2KD -	URE -	48H -			

99 % Pichia (Hansenula) anomala

Figure 4. Manual vitek lab report.

Table 2. Total ester yield in different mediums.

Medium	Total ester (g/l)
Barley saccharification mash 1	3.132
Barley saccharification mash 2	3.241
Wheat saccharification mash 1	3.570
Wheat saccharification mash 2	3.432
Corn saccharification mash 1	2.203
Corn saccharification mash 2	2.561
Sorghum saccharification mash 1	2.434
Sorghum saccharification mash 2	2.212
Rice saccharification mash 1	2.391
Rice saccharification mash 2	2.443

Table 3. Total ester yield of different culture methods.

Culture method	Total ester (g/l)
Static culture1	3.420
Static culture2	3.251
50 r/min Shake culture 1	2.383
50 r/min Shake culture 2	2.322
100 r/min Shake culture 1	2.116
100 r/min Shake culture 2	2.181
150 r/min Shake culture 1	2.080
150 r/min Shake culture 2	2.191

Table 4. Orthogonal test.

Test number	Brix	pH	Ethanol (%)	Temperature (°C)	Time (d)	Total ester (g/l)
1	6	6	4	28	4	2.271
2	8	4	0	28	3	2.587
3	10	4	4	30	5	2.220
4	12	6	0	30	2	0.930
5	6	5	0	20	5	1.530
6	8	3	4	20	2	0.194
7	10	3	0	25	4	2.978
8	12	5	4	25	3	2.198
9	6	3	6	30	3	0.264
10	8	5	2	30	4	3.679
11	10	5	6	28	2	0.606
12	12	3	2	28	5	0.814
13	6	6	2	25	2	0.862
14	8	4	6	25	5	2.094
15	10	4	6	20	3	0.194
16	12	6	2	20	4	2.724
Ⅰ	1.23	1.11	1.86	1.16		0.65
Ⅱ	2.21	1.77	2.09	2.03		1.31
Ⅲ	1.66	2.08	1.72	1.87		2.61
Ⅳ	1.55	1.69	0.80	1.73		1.66
R	1.71	1.80	1.61	1.70		1.56
The best level	2	3	2	2		3

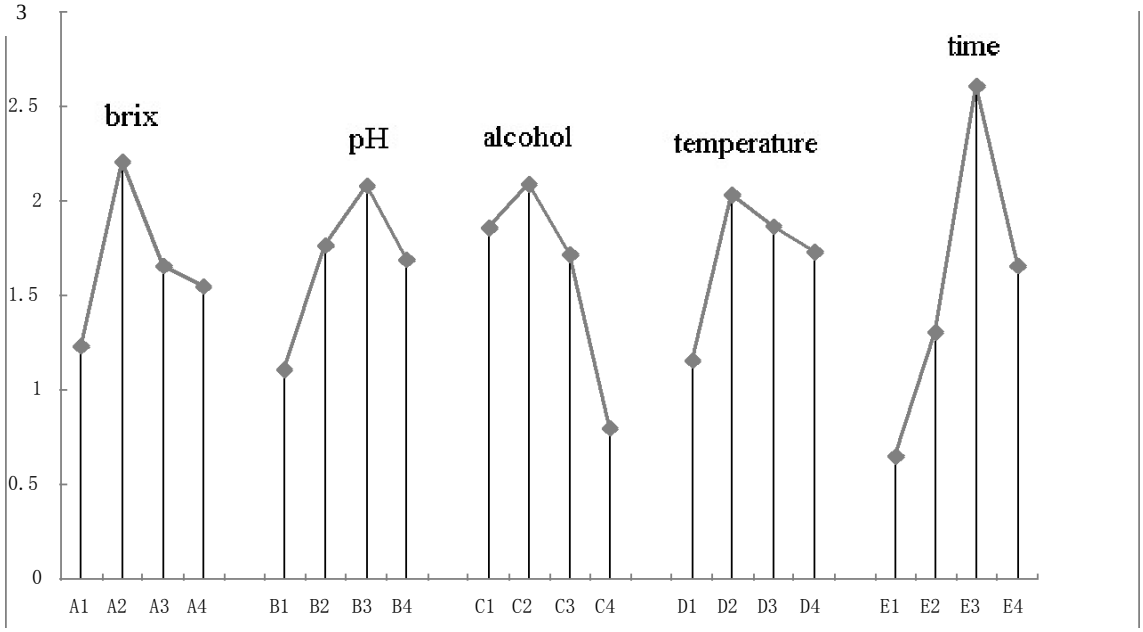


Figure 5. Change trend of the factors.

and it indicated that the optimum ester-producing conditions as follow: brix was 8°, pH was 5, temperature was 25°C, culture time was 4 days, and ethyl alcohol was 2%, ester production of Y1 was 4.812 g/l in this condition. When the change trend of the factors (Figure 5) was analyzed, it was learnt that in certain extent, sugar

content has promoting effect on ester yield, but high sugar content go against ester yield; although when the medium contained a little alcohol, it is of an advantage to ester yield. This might be that alcohol is the substrate of ester; and could promote the synthesis of ester when the content is little. However, high alcohol content can inhibit the growth of yeast and eventually lead to lower yield of ester. Ester yield increased rapidly in early fermentation, and on the fourth day, reached the peak, then, ester was quickly decomposed by ester decomposing enzyme with the fermentation.

Conclusion

A high yield of ethyl acetate yeast Y1 was screened, and the strain was identified as *Hansenula anomala*. It was seen that the best ester-producing medium for Y1 yeast was wheat saccharification mash, though static culture was better than shaking culture. However, the result of the orthogonal test indicated that the optimum ester-producing conditions for Y1 yeast are as follow: brix was 8°, pH was 5, temperature was 25°C, culture time was 4 days, and ethyl alcohol was 2%, total ester production of Y1 was 4.812 g/l in these conditions.

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