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Full Length Research Paper

Research on the effect of *Penicillium* sp. on Luzhou-flavor liquor

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***Penicillium* sp. was isolated from *Daqu* as a beginner strain. In order to investigate the role of *Penicillium* sp. in Luzhou-flavor liquor, *Penicillium* sp. was operated in Koji seeds and then simulated solid-state fermentation for liquor testing under different conditions. They included adding of different amounts of koji seeds and *Daqu*. The results showed that, when the amount of *Daqu* added was 20%, *Penicillium* sp. koji added increased from 0.5 to 2%, compared to blank control group. Liquor yield was reduced by 2.23% from 29.77%, total ester decreased by 1.70% from 37.25%, total acid increased by 6.57% from 12.41% and four esters had different degrees of decrease trend. While the amount of koji seeds added exceeded 1.0%, ethyl lactate content was higher than ethyl caproate content. When the amount of *Penicillium* sp. koji added was 1.0%, yield of liquor, total esters, total acid and the four esters were all enhanced with the increase in amount of *Daqu*.**

Key words: *Penicillium* sp., *Daqu*, Koji seeds, Luzhou-flavor liquor.

INTRODUCTION

Quality of *Daqu* was a major factor in the Luzhou-flavor liquor, as it is not only used for saccharification and fermentation but it also has fermented flavor enhancer effect. The quality of *Daqu* has direct impact on the production, quality and style of the liquor (Yao et al., 2003). As *Daqu* is prepared by natural inoculation of molds, yeasts and bacteria on the grains, its composition and quality depend heavily on experience, weather and geographical factors. This gives the characteristics to *Daqu* and liquors in their places of origin, and makes it difficult to reproduce these liquors in other places (Wang et al., 2008; Wang, 2008; Zhang et al., 2005). Ethyl caproate determines the main flavor and taste of *Luzhou-flavor* liquor and with significant proportion of total esters. Ethyl butyrate is another fragrant aroma component of the liquor, and low content of it would make the liquor to have fruit flavor; however, as the content of ethyl butyrate is higher than normal value, it would bring offensive smell to liquor and finally affect the liquor quality. Liquor presents apple and banana flavor while the content of ethyl acetate is high; otherwise it presents pear incense. Ethyl lactate content should be less than ethyl caproate in *Luzhou-flavor* liquor; otherwise it would effect the flavor and taste of the liquor (Wang et al., 2008a).

Penicillium sp. is a kind of microorganism frequently isolated from *Daqu* and with a certain proportion in the *Daqu* microbial populations. Some reports have said that *Penicillium* sp. is a harmful bacteria in *Luzhou-flavor* liquor, but the effect of *Penicillium* sp. in *Luzhou-flavor* liquor has rarely been studied. Therefore, in order to investigate the role of *Penicillium* sp. in *Luzhou-flavor* liquor, *Penicillium sp.* with cellulase and amylase was made into Koji seeds and then simulated solid-state fermentation for liquor testing under different conditions.

MATERIALS AND METHODS

Strains and medium

Penicillium sp. used in the experiment was isolated from *Daqu*, which was provided by Zhijiang Co., Ltd. *Penicillium* sp. was cultured on Potato Dextrose Agar medium (PDA) (Zhou, 2006), and Koji seeds was cultured on the solid medium containing: 20 g of

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Table 1. Different adding amounts of Koji seeds brewing test scheme.

Scheme	Koji seeds (%)	*Daqu* (%)	Raw materials (g)
1	0.5	20	600
2	1.0	20	600
3	1.5	20	600
4	2.0	20	600
Control	0	20	600

Table 2. Different adding amounts of *Daqu* brewing test scheme.

Scheme	Koji seeds (%)	*Daqu* (%)	Raw materials(g)
1	1.0	22	600
2	1.0	26	600
3	1.0	28	600
4	1.0	30	600
Control	0	20	600

Fupi, 18 ml of nutrient (Mandels, 1976). *Daqu* and raw materials used in liquor brewing were also provided by Zhijiang Co., Ltd.

Koji seeds cultured

Penicillium sp. was activated on PDA medium at 29°C for 7 days, and then its spores were washed into the solid medium with 1 ml sterilized saline water. The solid medium was cultured at 29°C for 5 to 6 days, and then dried at 35°C. It was then wrapped up with paper to be used later. The flask was shaken after 16 h, agitated when cultured after 30 h and patted to ensure the medium agglomerated as pie. The medium was obliquely placed in the flask space; medium was flipped up and down after cultured for 3 days. However, it should be ensured that the medium was not broken.

Liquor brewing

Liquor brewing was carried out at two different conditions and simulated solid-state fermentation for liquor brewing. Different amounts of Koji seeds added in the brewing scheme are shown in Table 1. In the experiment, 600 g raw materials was as inventory rating and 20% *Daqu* was added (Li, 2004), and then cultured at 30°C for 15 to 18 days. While different amounts of *Daqu* added for brewing test scheme are shown in Table 2. 600 g raw materials was as inventory rating in the test group and *Penicillium* sp. Koji added was 1.0%, and then fermented at 30°C for 15 to 18 days.

Analytical methods

The alcohol (c_1%,V /V) in the distilled liquor was determined by standard curve of ethanol (Tang, 2010), and then the alcohol (c%, m /m) and the liquor yield (which represent the abilities of transforming the utilized raw materials to alcohol) were calculated as follows:

The alcohol (c%, m/m) = the alcohol (c_1%, V/V) × ethanol density (0.7893, g /ml)

Liquor yield (96% ethanol, %) (Shen, 2007) = the alcohol (c%, m／m) × coefficient of ethanol transformed from 96 to 100%

(0.8411)／raw materials (g) ×100

Total ester content was determined by saponification method and indicated by ethyl acetate (Yi, 2008), while total acid content was measured with alkali titration and indicated by acetic acid (Yi, 2008). Four esters were determined by using a Gas Chromatograph equipped with a FID detector, and analyzed by amyl acetate internal standard (Shen, 2007; Cesar et al., 2009).

RESULTS AND DISCUSSION

Standard curve of ethanol

Figure 1 showed that linear regression equation of ethanol was as follows: $y = 1.4086x + 0.0084$, $R^2 = 0.9967$, and had good correlation. So it could be used to determine the alcohol (c_1%, V\V).

Liquor brewing results of different amount of Koji seeds added

It is seen in Figure 2, liquor yield and alcohol were all decreased while the amount of *Penicillium* sp. koji increased. And compared with blank control group, liquor yield reduced by 29.77% and alcohol deceased by 25.44%. Koji seeds added were up to 2%. Low liquor yield may be due to the presence of *Penicillium* sp. which inhibited the growth of *Rhizopus* studied in earlier research (unpublished). As we all know, *Rhizopus* is one of the main microbes which can produce glucoamylase. As a result, utilization of raw starch was reduced, furthermore liquor yield decreased.

It is shown in Figure 3, total esters deceased with the increase of *Penicillium* sp. koji; however, total acid increased. And the results showed that, when the amount of Daqu added was 20% , *Penicillium* sp. koji added increased from 0.5 to 2%, compared with blank control group; total ester decreased by 1.70% from 37.25% and

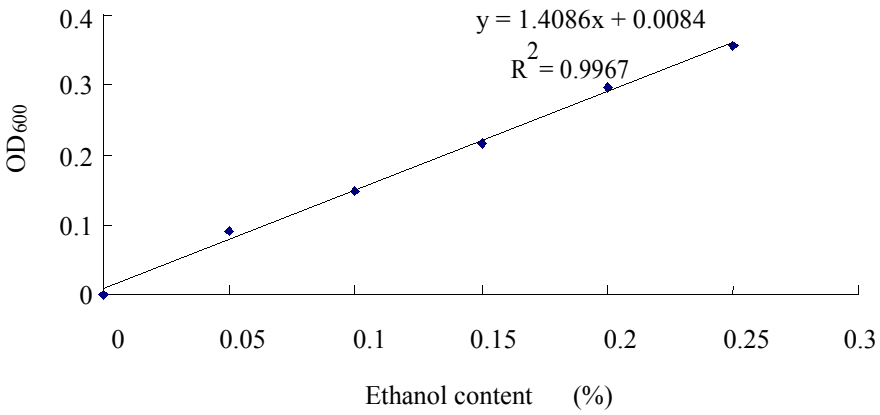

Figure 1. Standard curve of ethanol.

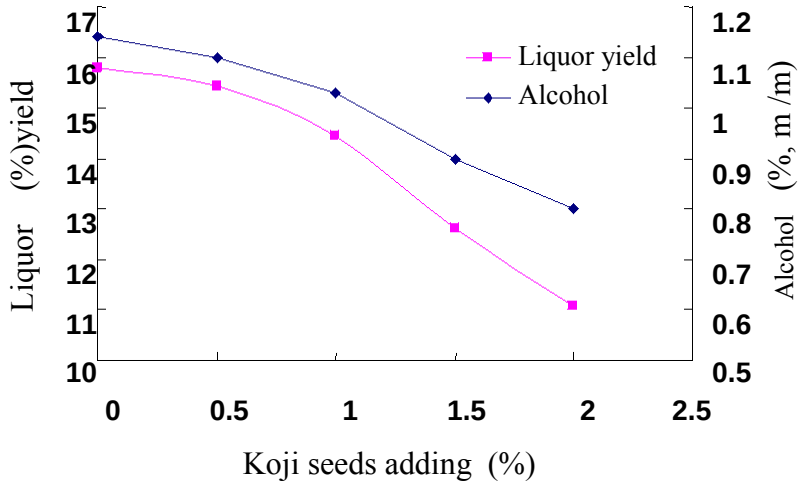

Figure 2. Alcohol and liquor yield of different *Penicillium* sp. Koji seeds adding amount.

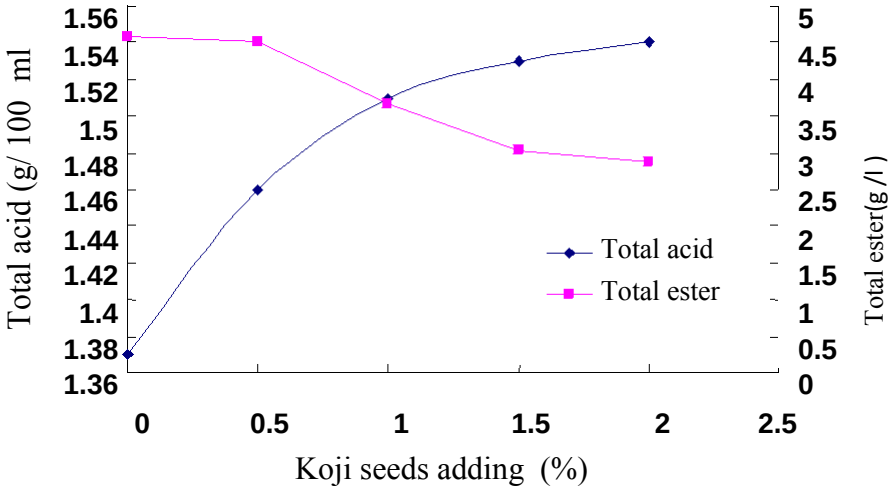

Figure 3. Total acid and total ester of different *Penicillium* sp. Koji seeds adding.

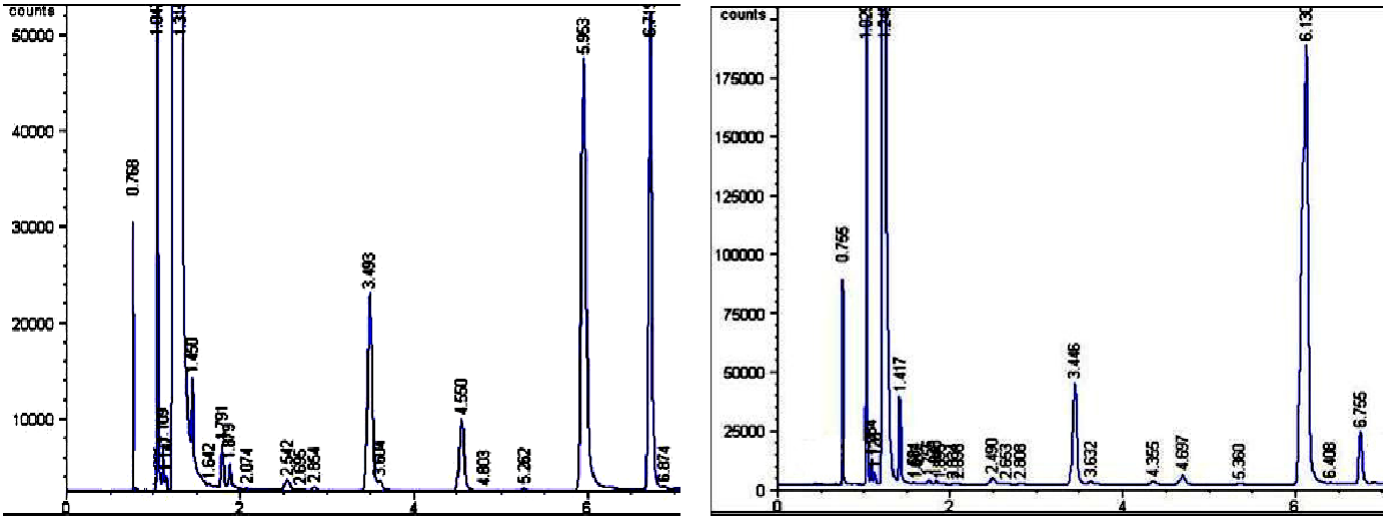

Figure 4. Ester content by gas chromatogram. A : blank control group; B: test group.

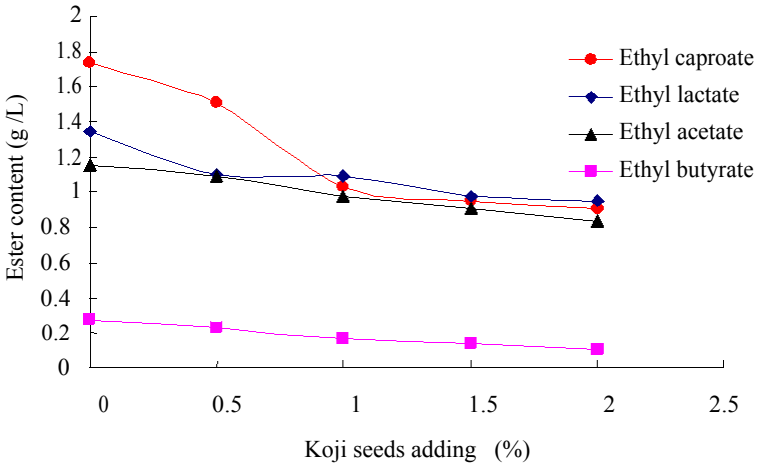

Figure 5. Content of four esters of different *Penicillium* sp. Koji seeds adding.

total acid increased by 6.57% from 12.41%. Generally speaking fermenting power reduced when acidity increased (Ciu, 2007), that is to say, fermenting power of *Daqu* reduced with the increase of *Penicillium* sp. koji. Acidity increase may be due to the performance of *Penicillium* sp. that could promote some acid bacteria such as *Lactobacillus* producing acid. Figure 4 shows ester content by gas chromatogram.

Four esters had different degrees of decrease trend when the amount of *Penicillium* sp. Koji increased (Figure 5), and the result was consistent with the conclusion of Figure 3. When *Penicillium* sp. Koji added increased to 2%, compared with the blank control group, ethyl caproate of test group reduced by 47.70%, ethyl lactate reduced by 29.63%, ethyl acetate decreased by 26.96%, ethyl acetate decreased by 59.26%. The concentration change of esters would affect the flavor and taste of the liquor, thus affecting the liquor quality. Ethyl lactate content was higher than ethyl caproate content when ethyl caproate increased to 1%. As ethyl caproate was the main flavor of the liquor, it finally affected the liquor flavor greatly. This may be because *Lactobacillus* had greater competition in the strong acidic environment; however, caproic acid bacteria had not suited the acidic environment, finally leading to concentration of ethyl caproate lower than ethyl lactate (Wang and Wang, 1994).

Liquor brewing results of different amounts of *Daqu*

Figures 6, 7 and 8 showed that alcohol, liquor yield, total

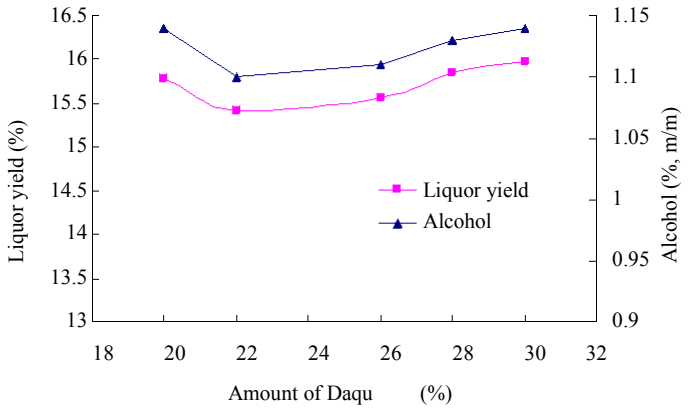

Figure 6. Alcohol and liquor yield of different amounts of *Daqu.*

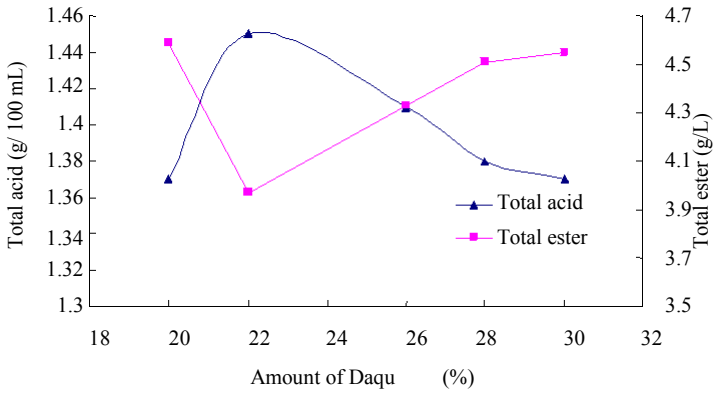

Figure 7. Total acid and total ester of different amounts of *Daqu*.

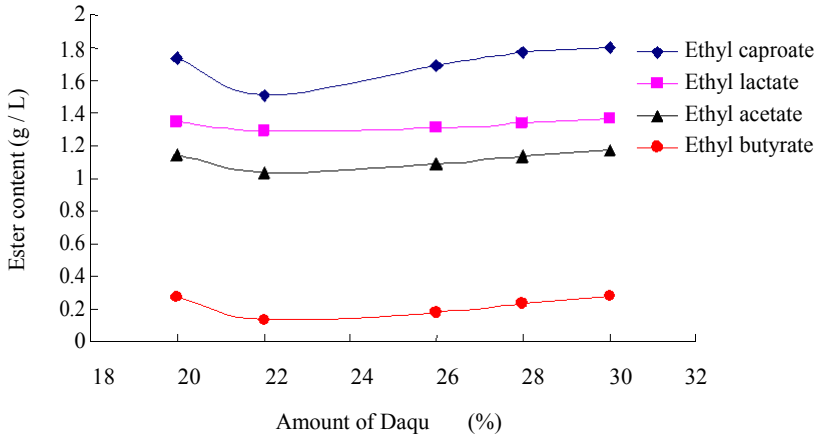

Figure 8. Content of four esters of different amounts of *Daqu*.

acid and total ester are all enhanced when the amount of *Daqu* increased. When *Penicillium* sp. koji added was 1.0% and the amount of *Daqu* was 28 to 20%, liquor yield, total esters, total acid and the four esters were all almost the same as the blank control. But obviously it would increase the production cost if the amount of Daqu

is increased

Conclusion

Liquor brewing test showed that *Penicillium* sp. is entirely harmful bacteria in *Luzhou-flavor* liquor. It does not only make liquor yield reduce and decrease total ester, it also make total acid increase, which finally affects fermentation (Ciu, 2007). Effects of *Penicillium* sp. on the liquor could reduce or disappear when the amount of *Daqu* increases; however, it would increase the production costs. As a result, it is necessary to control the existence of *Penicillium* sp. in *Daqu* during liquor production.

Penicillium sp. could grow well under lower temperature and damp conditions, and it was a strong inhibitor to other beneficial microbes that exist in *Daqu*. Heat released was less and temperature of environment grew slowly. This is because the mycelium was young and tender in the early period of *Daqu* cultured. Thus, it should be ensured that the temperature of *Daqu* cultured in early period was stable; as a result, *Aspergillus* could grow well so that *Daqu* quality could be stable and good. Otherwise, if the temperature was up and down, it would easily infect *Penicillium* sp. So, it was necessary to control the early temperature of *Daqu* cultured and the moisture of *Daqu* cultured.

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