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Study on the Interaction of Caproic Acid Producing Bacteria Communities from Different Source Pit Mud

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Abstract

The study aimed to investigate the differences in microbial growth and metabolites between co-cultivation and individual cultivation of JQ-1 and JQ-2 from different pit mud samples, with a focus on the interaction of caproic acid producing bacteria communities. The results demonstrated that co-cultivation of JQ-1 and JQ-2 significantly enhanced caproic acid metabolism, with caproic acid concentrations being 26.0% and 43.0% higher, respectively, than those observed in individual cultivation of JQ-1 and JQ-2. The dominant bacterium in JQ-1 was primarily *Rummeliibacillus*, while the dominant bacteria in JQ-2 were *Clostridium* sp. The microbial community structure of the co-cultivation was comparable to that of JQ-1, as determined by high-throughput sequencing analysis. However, the abundance of the dominant bacterial genera in the co-cultivation was increased, which indicated that *Rummeliibacillus* might be the crucial functional microorganism promoting caproic acid metabolism in the co-cultivation. Therefore, seven strains of bacteria were selected from JQ-1 and JQ-2 based on their high production of caproic acid. The highest yield of caproic acid was observed in *Rummeliibacillus suwonensis* SYS-1, with a production of 6.9 g/L after 12 days of cultivation. Following the co-cultivation experiment of SYS-1 and other strains, it was observed that SYS-1 had a promoting effect on the production of caproic acid when co-cultured with the majority of strains. The most significant effect was observed when SYS-1 was co-cultivated with *Enterococcus saccharolyticus* subsp. SYS-2. Furthermore, the hexanoic acid concentration reached 8.5 g/L, representing a 30.8% increase compared to single cultivation after 12 days of cultivation. This research contributes to the existing body of knowledge regarding the metabolism of *Rummeliibacillus suwonensis* SYS-1, and provides a framework for the industrial production of caproic acid using *Rummeliibacillus suwonensis* SYS-1.

Keywords: Caproic Acid Producing Bacteria; Individual Cultivation; Co-Cultivation; Rummeliibacillus Suwonensis

Introduction

The diversity of microorganisms present in pit mud plays a pivotal role in the formation of flavour compounds in Chinese strong-flavour baijiu [1,2]. Caproic acid-producing bacteria represent a significant microbial population in the brewing process of Chinese strong-flavor Baijiu [3], including *Clostridium*, *Bacillus*, *Pseudomonas*, etc [4-6]. This is because ethyl caproate is the principal flavour substance of Chinese strong-flavour Baijiu, and its concentration determines the quality of Baijiu [7,8]. Concurrently, the main metabolic product of the caproic acid-producing bacterial community is caproic acid, which serves as a precursor for the synthesis of ethyl caproate. Consequently, caproic acid-producing bacteria are regarded as a pivotal functional microorganism in the brewing process of Chinese strong-flavor Baijiu [9].

Microorganisms exhibit a high degree of diversity and are widely distributed in natural ecosystems, playing a pivotal role in maintaining ecological balance. In natural ecosystems, microorganisms typically interact with a multitude of strains and species in intricate ecological networks. During the processes of ecological succession and evolution, microbial interactions can influence the distribution of communities [10-12]. As shown in figure 1, Chinese strong-flavour baijiu is a product of pit mud fermentation. The structure and abundance of functional microbial communities in the microbial community inhabiting the pit mud are of great consequence to the fermentation process of strong-flavour Baijiu. The pit mud can be regarded as a small-scale microecosystem in which complex microbial interactions and metabolic processes are carried out. The research indicates that the yield of caproic acid produced by caproic acid bacteria when cultured alone was typically low. The majority of the existing high-yield caproic acid systems are mixed systems, and there may be extensive interspecific interactions between different species [13-15]. Currently, Chinese strong-flavour Baijiu generally exhibits the characteristics of a low ethyl caproate and a high ethyl lactate content. Consequently, an investigation into the interrelationship and utilisation of diverse caproic acid-producing bacterial communities is of paramount importance.

In this study, the caproic acid producing bacterial communities JQ-1 and JQ-2 from different sources were co-cultured and separately cultured, and the mutual mechanism of caproic acid producing strains in the microbial community and their adaptability in the ecosystem of Chinese strong-flavor Baijiu were explained through the analysis of microbial community changes, acid producing metabolic capacity, strain identification and microbial community adaptability, This study provides a theoretical reference for the interaction of caproic acid producing bacteria and its application in the production of Chinese strong liquor.

Materials and Methods

Microorganism and medium

JQ-1 and JQ-2, isolated from the pit mud of different Chinese baijiu factory, was used in this study. Clostridium diolis, Rummeliibacillus suwonensis, Lacrimispora celerrecrescens, Clostridium sp, Capriciproducens sp, Clostridium butyricum, Enterococcus saccharolyticus subsp were isolated from JQ-1 and JQ-2 and stored in China Centre for Type Culture Collection (CCTCC NO. M 2020881), was used in this study. Before the experiment, culture purity was tested through 16S rRNA sequencing (Ye 2020). Pre-cultivation was performed anaerobically in Ethanol Sodium (ES) medium at 34°C for 7 days, then the obtained culture broth was used as inoculum for fermentation. When cultured in solid medium, anerobic conditions were achieved by using the 2.5 L round bottom vertical anaerobic training bags (Qingdao Hi Tech Industrial Park Haibo Biotechnology Co. Ltd., China) and AnaeroPack (Mitsubishi Chemical Corporation, Japan). Because Caproic acid producing bacteria is an oxygen-tolerant microorganism (Fang et al. 2021), the anaerobic conditions provided by liquid submerged culture (set the filling capacity to 90%) can support its normal growth and metabolic activities. The ingredients in ES medium included (per 100mL): yeast extract 1 g, $MgSO_4$ $7H_2O$ 0.02 g, K_2HPO_4 0.04 g, $(NH4)_2$ SO_4 0.05 g, CH_3COONa 0.5 g, $CaCO_3$ 1 g, and 2 mL ethanol was added after sterilization. The medium was sterilized at 115°C for 30 min.

Fermentation and sampling

All fermentations were performed in 30 (mm)×200 (mm) test tubes and each sample containing 100 mL of fluid nutrient medium. JQ-1 and JQ-2 pre-culture (5% v/v) was inoculated into each test tube. Other strains were cultured in the same way. All the test tubes were sealed with sealing film, incubated statically at 34 ℃. Each experiment was performed in triplicates.

Fermentation performance test of JQ-1 and JQ-2

In order to study the caproic acid production of JQ-1 and JQ-2, JQ-1 and JQ-2 were added to the test tube containing 30 ml sodium ethoxide (ES) medium according to 5% inoculum volume, and were placed in 34 ℃ anaerobic culture for 12 days. The contents of acetic acid, butyric acid and caproic acid were determined at 0 d, 4 d, 8 d and 12 d, respectively.

Interaction of caproic acid-producing bacterial communities from different sources

In order to study the interaction between caproic acid producing bacterial communities enriched from different pit mud, JQ-1 and JQ-2 were simultaneously inoculated in ES medium with a volume ratio of 1:1 for co-cultivation, and compared with JQ-1 and JQ-2 cultured respectively, with a total inoculation amount of 5%. cultured for 16 days and regularly sample for detection of OD_{600}, and the concentration of acetic acid, butyric acid, ethanol, caproic acid, and microbial community changes.

Screening, identification and interaction of caproic acid producing bacteria communities in co-cultured ecosystem

In order to further explore the contribution of microorganisms in co-culture ecosystem, The isolated and purified strains were subjected to morphological observation and copper sulfate color test, and the strains were co-cultured. After 10 days of culture at 34 ℃, the caproic acid content was measured.

Analysis of cell growth and metabolites

2 mL Fermentation broth were taken each time from test tube to measure cell growth and the concentrations of metabolites. Cell growth was measured using a UV-spectrophotometer (U-V-1600PC, VWR, USA) at 600_{nm}. The pH was measured by a probe (MC125, Milwaukee Instruments, USA) inserted in the test tube top. Ethanol concentrations were measured by an SBA-biosensor (SBA-40D, Shandong Institute of Biological Sciences, China).

Qualitative analysis of caproic acid. Qualitative analysis of pit mud fermentation broth by Copper sulfate chromogenic method, take 2 mL of fermentation broth, add 2% copper sulfate solution 2 mL, dichloromethane 1 mL, set aside after full shaking and mixing, preliminary identification of caproic acid production based on color changes in the dichloromethane layer of the lower solution, the deeper the blue, the better the effect of producing caproic acid [16].

The concentrations of acetic acid, butyric acid, caproic acid were measured by gas chromatography-mass spectrometry (Agilent 5977B-7890B, USA) with a flame ionized detector (FID) and DB-WAX (30 m × 0.25 mm × 0.25 μm). 200 μL fermented broth was adjusted to pH 2.0 using 3 M H_2SO_4, and extracted with 2 mL CH_2Cl_2, followed by drying with anhydrous Na_2SO_4 [16]. The organic layer was filtrated through 0.22 μm filter and 1 μL resulting solution was then injected into the gas chromatograph. Helium (99.999%) was used as the carrier gas at the flow rate of 10.7 mL/min. The split ratio was 10:1. The temperature program was as follows: the oven temperature was held at 45°C for 1.5 min, and ramped at the rate of 8 °C/min to 225 °C and held for 1 min. The injection port, quadrupole mass filter and ion source were set at 260 °C, 150 °C and 230 °C, respectively. The electron ionization of FID was 70 eV. Scan range was m/z 50-550 [17].

Statistical analysis

All the determinations were performed in triplicate and the resulting mean value and standard deviation were displayed. The statistical significance of the data was evaluated via variance analysis by comparative averages (ANOVA).

Results

The results of fermentation performance test of JQ-1 and JQ-2

To study the caproic acid production of JQ-1 and JQ-2 in the ES medium, the concentration of acetic acid, butyric acid and caproic acid on day 0,4,8,12 were showed in Figure. 2.

Figure. 2 illustrates the concentrations of acetic acid, butyric acid and caproic acid at days 0, 4, 8 and 12. As illustrated in Figure. 2, the patterns of acetic acid, butyric acid and caproic acid during the fermentation process of JQ-1 and JQ-2 were largely similar, with the concentrations of acetic acid and butyric acid initially rising and then declining, while the content of caproic acid exhibited an upward trend. With regard to the trend in acetic acid content, the acetic acid content of JQ-1 was consistently lower than that of JQ-2, although the rate of decline was marginally faster than that of JQ-2. With regard to the trend of butyric acid content, it was observed that the butyric acid produced by JQ-1 began to be consumed on the fourth day, while that produced by JQ-2 began to be consumed on the eighth day. With regard to the trend of hexanoic acid content, on day 12, the hexanoic acid content in JQ-1 and JQ-2 was 9.4 g/L and 8.1 g/L, respectively. Furthermore, the rate of hexanoic acid production in JQ-1 was found to be significantly higher than that in JQ-2. This is because acetic acid and butyric acid are precursors for the formation of caproic acid. Consequently, the depletion of acetic acid and butyric acid implies the formation of caproic acid, which occurs earlier and in greater abundance in JQ-1 than in JQ-2.

The interaction results between caproic acid producing bacteria communities from different sources

In order to study the interaction between caproic acid-producing bacteria communities from different sources, two groups of caproic acid-producing bacteria, JQ-1 and JQ-2, enriched from pit mud of different distilleries were co-cultured in this study, and compared with their separate culture experiments. The experimental results are illustrated in Figure 3:

When JQ-1 and JQ-2 were co-cultured and individually cultured, the change trend of the OD600 value was found to be largely consistent, exhibiting an initial increase followed by a decline during the culture process. It is evident that the OD600 value of JQ-2 in monoculture is considerably lower than that of JQ-1 and the co-culture. The OD600 value of JQ-1 alone reached its maximum value of 0.88 on the fourth day, while the OD600 value of JQ-2 alone reached its maximum value of 0.41 on the eighth day. The OD600 value of JQ-1 and JQ-2 co-culture reached its maximum value of 0.66 on the eighth day (Figure. 3A).

When JQ-1 was cultured alone, the overall change trend of pH exhibited a biphasic pattern, initially increasing and then decreasing before returning to an elevated level. On the fourth day, the pH increased to 6.6, then rapidly decreased to 5.7, and then returned to 6.0. Thereafter, the pH exhibited a gradual stabilisation. When JQ-2 was cultured alone, there was an initial increase in pH, followed by a subsequent decrease. On the fourth day, the pH increased to 6.4 and then gradually decreased to 5.8, before stabilising. The change trend of pH in the co-culture was identical to that observed in the JQ-1 culture. On the fourth day, the pH increased to 6.4, then rapidly decreased to 5.9, and then rapidly recovered to 6.5, and then gradually stabilized(Figure. 3B).

According to the report, the pKa (acid dissociation constant) of butyric acid is approximately 4.82. At pH levels ranging from 5 to 5.5, a significant portion of butyric acid exists in an undissociated form. The undissociated form of butyric acid is highly lipophilic, easily penetrating cell membranes, and exhibits strong toxicity to microorganisms [26]. This toxicity can inhibit the synthesis of caproic acid. However, at pH levels between 6 and 6.5, butyric acid primarily exists in a dissociated form, which is less toxic to microorganisms. Consequently, the inhibition of caproic acid synthesis is relieved. Notably, during the co-culture process described, the pH of the entire fermentation remains within the range of 6-6.5. This pH range is more favorable for caproic acid formation(Figure. 3B).

When JQ-1 and JQ-2 were cultured individually and in co-culture, the trend of ethanol concentration in the fermentation broth was consistent. Initially, there was a rapid decrease in ethanol concentration over the first four days, followed by a gradual decline thereafter. At the end of the fermentation process, the ethanol concentrations were measured as 3.6 g/L for the co-culture, 5.2 g/L for JQ-1, and 5.4 g/L for JQ-2, respectively. Notably, the coculture showed the lowest ethanol con-

centration, indicating that the ethanol consumption rate of microorganisms in the coculture system was higher (Figure. 3C).

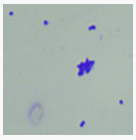
Acetic acid and butyric acid exhibited a pattern of initially increasing and then decreasing when JQ-1 and JQ-2 were cultured respectively and co-cultured.The changing trend of butyric acid and acetic acid was consistent with the study of P. San-Valero [18]. According to the reverse β-oxidation reaction, ethanol was oxidized to produce acetic acid and acetyl--CoA. Acetyl-CoA combined with acetic acid to generate butyric acid. Then butyric acid combined with acetyl-CoA to generate hexanoic acid [13,19]. The initial increase in acetic and butyric acid concentrations, followed by a subsequent decrease, can be attributed to the synthesis of hexanoic acid from ethanol, whereby butyric and acetic acids act as intermediates (Figure. 4).

At the end of fermentation, the concentrations of acetic acid and butyric acid were 0.26 g/L and 0.97 g/L for JQ-1 cultured alone, 0.3 g/L and 1.25 g/L for JQ-2 cultured alone, and the lowest concentrations of acetic acid and butyric acid were 0.18 g/L and 0.72 g/L for the co-culture, respectively. The decrease in acetic acid and butyric acid concentrations corresponded to the formation of caproic acid (Figure. 3D, Figure. 3E).

The concentration of caproic acid in both JQ-1 and JQ-2 exhibited an initial increase followed by a gradual stabilization as fermentation time progressed, whether they were cultured respectively or co-cultured. Within the first 4 days, JQ-1 demonstrated the highest production of caproic acid, which corresponded to the highest OD_{600} value, highest pH, and highest ethanol consumption observed on the fourth day. This pattern is consistent with the reverse β-oxidation pathway, which is involved in the metabolic generation of caproic acid by caproic acid bacteria [18,27]. However, after 8 days of fermentation, the caproic acid production was gradually surpassed by the co-culture. At the end of fermentation, the co--culture exhibited the highest concentration of caproic acid at 11.9 g/L, while JQ-1 and JQ-2 alone cultures showed concentrations of 9.4 g/L and 8.3 g/L, respectively (Figure. 3F).
Rummeliibacillus in JQ-1 dominated the entire fermentation process and increased with the fermentation time. The abundance of *norank_f_Ruminococcaceae* slowly decreased after gradually increasing from 0 to 8 days. *Lysinibacillus* dominated in the early stages of fermentation, but its abundance rapidly decreased after 4 days. The abundances of *Clostridium_Sensu_Stricto_1* and *Clostridium_Sensu_Stricto_18* significantly decreased on the 4th day, then increased significantly on the 8th day, before gradually decreasing thereafter. *Clostridium_Sensu_Stricto_12* and *Clostridium_Sensu_Stricto_10* gradually increased from 0 to 4 days and then slowly declined. The abundance of *unclassified-f-Ruminococcaceae* started to decrease slowly after gradually increasing from 0 to 8 days. *Lachnochlostridium* had a relatively small proportion throughout the fermentation process, with little change in abundance from 0 to 4 days, followed by a decline(Figure. 5).

The *Clostridium* genus in JQ-2 dominates the entire fermentation process, *Clostridium_Sensu_Stricto_12* holds the main advantage in the early stage of fermentation, with a significant decrease in abundance from 0 to 4 days, increased rapidly from 4 to 8 days, and gradually began to decline slowly; *Clostridium_Sensu_Stricto_1*, *Clostridium_Sensu_Stricto_18* and *Clostridium_Sensu_Stricto_1*. the Abundance shows a trend of increasing first and then decreasing during the fermentation process. Among them, The abundance of *Clostridium_Sensu_Stricto_1* and *Clostridium_Sensu_Stricto_18* has increased more significantly. The abundance of *norank_ f_Ruminococcaceae* and *unclassified_ f_Ruminococcaceae* shows an overall upward trend; *Lachnochlostridium* has a relatively small abundance in the early stage of fermentation, but gradually increases and occupies a certain advantage in the later stage (Figure. 5).

Table 1: Sequence homology alignment and acid production results of 7 strains of caproic acid bacteria

Name	Strain	Colony morphology	Microscopic results	Caproic acid content（g/L）	Copper sulfate color results
SYS-1	*Rummeliibacillus suwonensis*			6.9	++
SYS-2	*Enterococcus saccharolyticus* subsp			5.5	++
SYS-3	*Caproiciproducens* sp			1.3	+
SYS-4	*Clostridium* sp			0.2	-
SYS-5	*Clostridium diolis*			0	+
SYS-6	*Clostridium butyricum*			0	-
SYS-7	*Lacrimispora celerecrescens*			2.3	+

Note: "++" indicates that a lot of caproic acid is production, "+" indicates caproic acid production, "-" indicates no caproic acid production.

During co-cultivation, the genus *Rummeliibacillus* dominates the entire fermentation process, showing a trend of first increasing and then decreasing. The abundance of other microorganisms is relatively small, and the trend is as follows: The overall fermentation process abundance of *unclassified_f_Ruminococcaceae* and *norank_f_Ruminococcaceae* did not change significantly, *Clostridium_Sensu_ Stricto_10* and *Clostridium_Sensu_Stricto_1* genus significantly decreased from 0 to 4 days, and then gradually increased; *Clostridium_Sensu_Stricto_10* and *Clostridium_Sensu_Stricto_1* genus decreased significantly in 0-4 days, and then increased gradually; *Clostridium_Sensu_Stricto_18* abundance decreased significantly from 0 to 4 days, and then increased gradually; *Lachnoclostridium* was low in the whole fermentation process, and increased significantly in 0-4 days, and then decreased gradually (Figure. 5).

Isolation, identification, and interaction results of caproic acid bacteria communities in co-cultured ecosystems

Because JQ-1 and JQ-2 co-culture has a positive role in promoting caproic acid production by caproic acid producing bacteria, in order to further explore the contribution of these bacteria, a total of 7 strains were selected from the co culture system for molecular identification, and the total DNA was extracted, then the 16S rRNA gene was amplified by PCR and sequenced. They were named SYS-1,SYS-2 to SYS-7,and the 16S rRNA gene sequence of the tested bacteria was submitted to NCBI for online comparison and copper sulfate color analysis (Table 1), there were *Lacrimispora celerecrescens*, *Enterococcus saccharolyticus* subsp, *Clostridium diolis*, *Clostridium* sp, *Caproiciproducens* sp, *Clostridium butyricum*, *Rummeliibacillus suwonensis*.

When *Rummeliibacillus suwonensis* co-cultured with other strains (*Clostridium* sp, *Clostridium butyricum*, *Enterococcus saccharolyticus*, *Clostridium diolis*, *Lacrimispora cellerecrescens*, *Capriciproducens* sp). Results as shown in the figure, co--culture of *Rummeliibacillus suwonensis* with most strains (*Clostridium* sp, *Clostridium butyricum*, *Enterococcus saccharolyticus*, *lacrimispora celleracrescens*) can promote caproic acid metabolism, but the effect is the most significant when co-cultured with *Enterococcus saccharolyticussub*, with caproic acid concentration of 10.5g/l, which is 50% higher than that when cultured alone. *Rummeliibacillus suwonensis* belongs to the genus *Rummeliibacillus*. This result once again proves that JQ-1 and JQ-2 co-culture promoting caproic acid metabolism is directly related to the increase and stability of *Rummeliibacillus* genus(Figure. 6).

Discussion

In this study, the caproic acid producing bacterial communities JQ-1 and JQ-2 from different sources were co-cultured and separately cultured, and the mutual mechanism of caproic acid producing strains in the microbial community through the analysis of microbial community changes, acid producing metabolic capacity, strain identification and microbial community adaptability, and focused on the contribution of each strain in the co-culture ecosystem.

The above results indicate that the highest concentration of caproic acid in JQ-1 was observed during the first four days of fermentation. This occurred concurrently with the highest values of OD600, pH, and ethanol consumption of JQ-1 on day 4, which is consistent with the involvement of caproic acid-producing bacteria in the trans-β-oxidation pathway of caproic acid production (Figure. 3). Eight days later, the caproic acid production of the co-cultured bacteria exhibited a gradual increase, exceeding that of JQ-1. At the conclusion of the fermentation process, the highest concentration of caproic acid was observed in the co-cultured bacteria, reaching a concentration of 11.9 g/L. In comparison, the JQ-1 and JQ-1 produced a greater quantity of caproic acid than the JQ-1. In comparison, the individual cultures of JQ-1 and JQ-2 exhibited hexanoic acid concentrations of 9.4 g/L and 8.3 g/L, respectively. The concentration of hexanoic acid in the co-cultures was markedly higher than that observed in the individual cultures. This phenomenon suggests that the observed differences may be mainly attributed to changes in the microbial community.

According to the sequencing analysis results, the microbial communities in JQ-1 and JQ-2 are significantly different(Figure. 5). The dominant bacteria in JQ-1 are mainly *Rummeli-*

ibacillus, supplemented by *Clostridium*. The dominant bacteria in JQ-2 are mainly *Clostridium*. However, when subjected to the same culture conditions, the caproic acid yield of JQ-1 was markedly higher than the caproic acid yield of JQ-2. This is because R*ummeliibacillus*, on the one hand, is involved in the production of caproic acid itself [28]. On the other hand, *Rummeliibacillus* is a facultative anaerobic bacterium with certain aerobic capacity. It can not only consume oxygen in the early stage and rapidly reproduce, but also provide an anaerobic environment for *Clostridium*, which is more conducive to promoting the formation of caproic acid, Moreover, the high abundance of *Clostridium* in JQ-2 may lead to the low pH of fermentation broth, Previous studies have revealed that the neutral acidic environment was more suitable for caproic acid-producing bacteria to generate caproic acid [20,21] also reported that the rate of caproic acid formation was reduced at a pH below 5.5 and its production was completely inhibited below pH 5. further inhibiting the caproic acid formation pathway of *Clostridium*, which corresponds to the low pH of JQ-2 fermentation process.

Following the co-cultivation of JQ-1 and JQ-2, a greater similarity was observed in the distribution of microbial communities in JQ-1 and the co-culture (Figure. 5). The co-cultivation of JQ-1 and JQ-2 resulted in an increase and stability in the dominant bacterial genus *Rummeliibacillus* in JQ-1, while the genus *Clostridium* also increased; however, the overall change was not significant. The only discernible difference was that the abundance of *Lysinibacillus* in JQ-1 during the initial fermentation stage was considerably higher than that observed in the co-culture. Both *Lysinibacillus* and *Rummeliibacillus* are capable of producing caproic acid [22]. However, *Rummeliibacillus* is a facultative anaerobic bacterium with certain aerobic capacity. It can be hypothesised that this microorganism may primarily utilise oxygen for reproduction during the initial stages of fermentation, which may prove to be an obstacle to the production of caproic acid. In the initial stages of fermentation, the genus *Lysinibacillus* in JQ-1 is likely the primary microorganism responsible for the production of caproic acid. This is the underlying reason for the higher yield of caproic acid and accelerated ethanol consumption observed in JQ-1 compared to the co-cultivation system.

The above results showed that compared with JQ-1 and JQ-2, co-culture was helpful in increasing the abundance and stability of *Rummeliibacillus suwonensis*, and promoted the interaction between *Rummeliibacillus suwonensis* and other microorganisms (*Clostridium diolis*, *Lacrimispora cellerecrescens*, *Clostridium* sp, *Caproiciproducens* sp, *Clostridium butyricum*, subsp), thereby increasing the concentration of caproic acid by 21% and 30%, respectively. However, the interaction mechanism between microorganisms in the co-culture ecosystem needs to be further analysed.

Through the liquid fermentation experiment for the first time, this study verified that caproic acid producing bacteria from different sources can promote each other, and determined that *Rummeliibacillus suwonensis* is the main functional microorganism that promotes the interaction, which provides a certain reference for the follow-up study on the interaction mechanism between *Rummeliibacillus suwonensis* and other microorganisms.

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Data Availability

The datasets generated during and/or analyzed during the current study are available fromthe corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

Ethical Statements

This article does not contain any studies with human participants or animals performed by any of the authors.

Author Contributions

WCF and SLF conceived and designed the study. WCF and WWY conducted experiments. XJ and YXX analyzed data. HC and BL contributed with the analytical tools. WCF and WWY wrote the original manuscript. ZHM and SLF contributed to the review and editing of the manuscript. MBC and SLF contributed to project supervision and advice. All authors read and approved the manuscript.

Figure Legends

Figure 1: The fermentation process of Chinese strong flavor Baijiu.

Figure 2: Fermentation performance test of JQ-1 and JQ-2.

Figure 3: Results of the interaction between caproic acid production communities from different sources: OD_{600} (A), pH (B), ethanol content (C), acetic acid (D), butyric acid (E), hexanoic acid (F).

Figure 4: The metabolic pathways of caproic acid producing bacteria when using ethanol.

Figure 5: The distribution of the microbial community in the fermentation broth.

Figure 6: Interaction results of caproic acid strains in co-cultured ecosystems.

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