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影响鲜湿米粉食味品质微生物的分离与鉴定

Isolation and identification of microorganism affecting eating-quality in fermented fresh rice noodle

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摘要:从鲜湿米粉发酵液中分离并鉴定影响其食味品质的微生物菌株,以制备发酵剂推进米粉生产的工业化进程。首先从传统米粉发酵液中,分离得41株酵母菌和60株乳酸菌(LAB),并对其胞外酶活性(过氧化氢酶、β-葡萄糖苷酶、脂肪酶、α-淀粉酶、蛋白水解酶)、不同pH值和温度下的生长和酸化能力等技术特征进行分析,共筛选出11株酵母菌株和19株乳酸菌。然后通过对酵母菌的内转录间隔区序列和乳酸菌的16S rRNA基因鉴定,经基因型和系统发育树分析表明:3株酵母菌和4株乳酸菌。采用这7株菌对大米进行控制发酵制备鲜湿米粉,结果表明,乳酸菌中 *L. plantarum* CSL 23发酵制备的米粉拉伸强度、硬度、内聚性和吸水率最高,黏附性和蒸煮损失最低;酵母中 *S. cerevisiae* CSY 13发酵的米粉品质更好。因此,2株菌具有用来制备鲜湿米粉专用发酵剂的潜力,为规模化控制发酵生产鲜湿米粉提供试验依据。

关键词:发酵米粉;酵母菌;乳酸菌;基因型;发酵剂

Abstract: Microorganism strains as the starter culture for the manufacture of fermented rice noodles were selected. A total of 41 yeasts and 60 lactic acid bacteria (LAB) were isolated from the traditional fermentation of rice noodles, and the enzymatic activities (catalase, β-glucosidase, lipase, α-amylase, proteolytic enzyme), growth under varied pH and temperatures, and acidification capability were characterized. Principal component analysis indicated that 11 isolates of yeast, and 19 isolates of LAB exhibited potential technological traits. After identification using internal transcribed spacer sequence for yeasts and 16S rRNA gene for LAB, the isolates were grouped into three yeast isolates and four LAB isolates by their genotypes and phylogenetic trees. Noodles fermented by a LAB isolate from the *Lactobacillus plantarum* group (CSL 23) led to higher tensile strength, hardness, cohesiveness and cooked weight, and lower adhesiveness and cooking loss, than that by the other LAB isolates. Noodles fermented by a yeast isolate from the *Saccharomyces cerevisiae* group (CSY 13) were of better qualities than that by the other yeast isolates. Thus, these two isolates were the potential starter cultures for preparing rice noodles. The results would provide a guidance for maintaining consistent quality during large-scale production of fermented rice noodles.

Keywords: fermented rice noodle; yeast; lactic acid bacteria; genotype; starter culture

Fermented rice noodles are a popular staple food in South China and Southeast Asia. They are generally manufactured by fermenting *indica* rice naturally. This is then ground to a slurry, steamed to form a gel then finally extruded into different types of vermicelli (Fu, 2008). For traditional production, fermentation occurs spontaneously when whole polished rice grains are steeped in water at ambient temperature (10～40 ℃) for 2～6 d then fermented by the indigenous microbiota. The physicochemical changes in the rice induced by microbial fermentation have been shown to improve their texture and mouthfeel (Tan, Li, Tan, 2009).

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Rice fermentation relies on a complex microflora where both functional microorganisms and spoilage or pathogenic microorganisms, such as lactic acid bacteria (LAB) and yeasts, can breed (Lu, Peng, Cao, Tatsumi, & Li, 2008). This multiple ecosystem leads to variable and unstable qualities and thus a short shelf-life for the rice noodles.

Using a starter culture to initiate the rice fermentation process is a well-practiced and attractive method for producing food with consistent qualities on a large scale. A starter culture should contain at least one definite microbial preparation with a high cell density, which should adapt well to the environment of rice fermentation and thereby create an unfriendly environment for undesirable spoilage and pathogenic microorganisms (Hong, Chen, Liu, Wu, Tan, Xie, Xu, Zou, Yu, Wang, & Qin, 2016). The starter culture should also improve the shelf-life, sensory quality, and safety of the product (Rubio, Jofré, Martín, Aymerich, & Garriga, 2014). The development of effective starter cultures relies on isolating and identifying the predominant microorganisms having the best technological, probiotic, and economic characteristics (Zanirati, Abatemarco, Sandes, Nicoli, & Nunes, 2015). Yeasts and LAB both have a long history of safety and technological use in fermented foods. For example, yeasts are widely used as a starter in manufacturing pancakes, bread, and in amylolytic fermentation (Aidoo, Rob Nout, & Sarkar, 2006). LAB are also used as starter cultures for manufacturing many fermented foods, particularly dairy products such as cheese and fermented milks. LAB fermentation can generate organic acids, carbon dioxide, exopolysaccharides and diacetyl that contribute to the texture, aroma and shelf-life of fermented foods (Leroy & De Vuyst, 2004; Settanni, Ventimiglia, Alfonzo, Corona, Miceli, & Moschetti, 2013).

The aim of the study is to evaluate the effect of specific microorganisms after isolation on the quality of rice noodles, to identify the predominant microorganisms present in the spontaneously fermented liquid, and to characterize these microorganisms using their technical properties. This will enable the selection of the most suitable starter cultures for producing fermented rice noodles.

1 Material and methods

1.1 Isolation of microorganisms

In three factories (Hunan, China), untreated rice grains were steeped in water at 40 ℃ for 3.5 d to ferment spontaneously. We sampled supernatants from the process after 0, 1, 2, 3, and 3.5 d of fermentation. The samples were then serially diluted with a sterile saline solution (0.9% NaCl) then plated on two types of medium:

(1) Yeast extract peptone dextrose (YPD) agar plates (yeast extract, 10 g/L; peptone, 20 g/L; dextrose, 20 g/L; agar, 18 g/L), with 0.2 g/L chloramphenicol (Ruibio, Darmstadt, Germany), were incubated at 30 ℃ for 48 h before isolation and evaluation of the yeasts. Yeast colonies showing the typical cell appearance under a microscope were randomly selected and labeled with a code (CSY 01 to CSY 41). After purifying five times with successive sub-culturing, the yeasts were maintained on YPD agar slant culture-medium, stored at 4 ℃ and transferred monthly.

(2) MRS agar plates, with 0.1 g/L cycloheximide (Sigma-Aldrich, St Louis, MO, USA), were incubated at 30 ℃ for 48 h before isolation and evaluation of lactic acid bacteria. LAB were randomly selected and labeled with a code (CSL 01 to CSL 60). After purifying five times with successive sub-culturing, LAB were maintained on MRS agar slant culture-medium with added $CaCO_3$ at 20 g/L then stored at 4 ℃ and transferred monthly.

Before each test, the isolated LAB and yeasts were grown up to 7 lg CFU/mL in MRS and YPD broth, respectively.

1.2 Technological characterization

1.2.1 Screening for enzyme activity The activities of the enzymes were tested as follows: catalase as described by Gamero-Sandemetrio, Gómez-Pastor, & Matallana (2013), β-glucosidase as described by Caridi, Pulvirenti, Restuccia, Sidari, Mediterranea, Francesco, Agrarie, & Emilia (2005), lipase as described by Kouker & Jaeger (1987), α-amylase as described by Strauss, Jolly, Lambrechts, & Van Rensburg(2001), and proteolytic enzymes as described by Saran, Isar, & Saxena (2007). The enzymatic activity was expressed as: "−" (no activity), "+" (weak activity) and "++" (strong activity).

1.2.2 Growth at different pH values and temperatures

Sterile tubes, containing 10 mL broth, were adjusted to pH values of 3.0, 4.0, 5.0, 6.0, and 7.0 using HCl, inoculated with approximately 5 lg CFU/mL of yeasts or LAB then incubated at 30 ℃ for 48 h. In addition, sterile tubes containing 10 mL of broth (pH 6.0) were inoculated with approximately 5 lg CFU/mL of yeasts or LAB then incubated at 10, 25, 40 ℃ for 48 h.

Microbial growth at 48 h was measured after being diluted 100 times at 600 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (UV-2800; Unico, Shanghai, China). The absorbance value was used to define the growth ability (GA) of isolated isolates under different conditions, with GA values classified as follows:

$GA < 0.1$: no growth ability;

$0.1 < GA < 0.4$: weak growth ability;

$0.4 < GA < 0.7$: moderate growth ability;

$GA > 0.7$: strong growth ability.

1.2.3 Acidification capability in sterile rice extracts (SRE)

Sterile rice extract (SRE) broth was prepared as follows: 200 g *indica* rice was added to 800 mL sterile water, boiled for 20 min so that the rice was well cooked, then filtered using eight layers of gauze. The filtered liquor was diluted with sterile water to 1 000 mL then sterilized at 121 ℃ for 20 min for use in the subsequent experiments.

The yeasts and LAB cultures, grown overnight in YPD and MRS broth, respectively, were collected by centrifugation at 4 500 × *g* for 5 min, washed with Ringer's solution, then re-suspended in the same solution to give an absorbance of 1.00 at 600 nm measured using a UV-Vis spectrophotometer (UV-2800) (Alfonzo, Ventimiglia, Corona, Di Gerlando, Gaglio, Francesca, Moschetti, & Settanni, 2013). A 2 mL sample of the cell suspension was incubated in 150 mL of SRE at 30 ℃. The pH was measured after 0, 4, 8, 12, 24, 36, and 48 h of incubation. The acidification capability of each microorganism was calculated using one-variable linear regression to assess the relationship between the time and the pH value. The absolute values of the regression coefficient (r) represent the acidification capability as follows:

$r < 0.03$: no acidification capability;

$0.03 < r < 0.04$: weak acidification capability;

$0.04 < r < 0.05$: moderate acidification capability;

$r > 0.05$: strong acidification capability.

1.3 **Genotypic identification**

1.3.1 ITS (Internal Transcribed Spacer) sequence analysis of yeasts The genomic DNA of the selected yeasts was extracted using an extraction kit (Sangon Biotec, Shanghai, China) according to the manufacturer's procedure. The ITS-5.8s-ITS regions of the genomic DNA were amplified from the total DNA using the universal forward primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and the reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Bellemain, Carlsen, Brochmann, Coissac, Taberlet, & Kauserud, 2010). The PCR was performed in a 50 μL reaction system containing 5 μL 10 × PCR buffer, 0.5 μL dNTPs (10 mmol/L each), 10 ng genomic DNA, 0.5 μL Forward Primer (50 mmol/L), 0.5 μL Reverse Primer (50 mmol/L), 0.5 μL 5 U/μL Taq DNA Polymerase (Thermo, Shanghai, China). The PCR conditions were as follows: 95 ℃ for 5 min; 35 cycles at 95 ℃ for 30 s, 54 ℃ for 30 s, 72 ℃ for 40 s; and finally, 72 ℃ for 10 min. The PCR product was verified by agarose gel electrophoresis and purified using the Gel Extraction Kit (Sangon Biotec). The PCR-amplified DNA for each sample was sent to a commercial company (Sangon Biotec) for sequencing. The sequences obtained were compared with reference sequences from the GenBank Database using the BLAST program so that the most similar relative sequence could be determined. All sequences were aligned using Clustal X2.1 software (www.clustal.org). The phylogenetic tree was constructed by the MEGA program (version 6.06) using the maximum likelihood method with the Tamura-Nei model (www.megasoftware.net).

1.3.2 16S rRNA gene sequence analysis of LAB The 16S rRNA gene regions of the selected LAB were amplified from the genomic DNA using the universal forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-TACGGTTACCTTGTTACGACTT-3′) (Heilig, Zoetendal, Vaughan, Marteau, Akkermans, & DE VOS, 2002). The PCR conditions were as follows: 95 ℃ for 5 min; 35 cycles at 95 ℃ for 30 s, 51 ℃ for 30 s, 72 ℃ for 90 s; and finally, 72 ℃ for 10 min. The other procedures were the same as those for the ITS sequence analysis assay of yeasts.

1.3.3 Nucleotide sequence accession numbers The sequences in this study were deposited in the GenBank database: 16S rRNA sequences under the accession numbers, KT800388-KT800406 and ITS sequences under the accession numbers, KT800407-KT800417.

1.4 **Application in the production of rice noodles**

1.4.1 Preparation of rice noodles Yeasts and LAB were inoculated in YPD and MRS broth, respectively, then incubated overnight at 40 ℃. Under aseptic condition, 5 mL of the overnight cultures were transferred into 500 mL Erlenmeyer flasks containing 300 mL sterile water and 250 g early *indica* rice. The flasks were sealed with eight layers of gauze and maintained at 40 ℃ for 48 h. Meanwhile, rice was steeped in tap water with no starter culture and held at 40 ℃ for 48 h to serve as the control group.

The fermented rice granules were then freeze dried in a vacuum freeze-drying oven (Beijing Boyikang Instruments Co., Ltd., Beijing, China), milled using a universal grinder (Taisite, Tianjin, China), then passed through a 100 mesh sieve. The rice noodles were prepared as described by Singh & Kaur (2010) with minor modifications. Briefly, 5 g of the dry rice flour was mixed with water at a ratio of 1 ∶ 7 (g/mL), then cooked for 5 min in a water bath at 95 ℃. Then, 95 g of dry rice flour was mixed with the cooked flour and kneaded thoroughly by hand for 5 min to obtain a uniform dough with a moisture content of about 40%. The surface of the dough was gelatinized by steaming for 90 s, then extruded into vermicelli (1.5 mm diameter) using a cylindrical hand extruder. The extruded noodles were immersed in boiling water for 30～40 s, cooled by immersion in tap water for 3 min, and then drained for 2 h.

1.4.2 Tensile strength and texture profile analysis (TPA)

Semi-dry rice noodles were boiled in water for 3 min, cooled in tap water for 1 min, then drained and evaluated immediately. The tensile strength and texture profile analysis (TPA) of the noodles were evaluated using a TA-XT plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK). The tensile strength of the noodles was tested at a speed of 3.0 mm/s. The textural properties of the cooked noodles were measured using a P/36R probe with the following parameters: deformed 50% at a speed of 1 mm/s then paused for 5 s. The textural characteristics, hardness, adhesiveness, and cohesiveness, were obtained. Ten tests were made on each sample.

1.4.3 Determination of cooking qualities The cooking qualities were determined as described by Cham & Suwannaporn (2010). Briefly, 5 g of semi-dry rice noodles were boiled in 150 mL distilled water for 6 min, drained for 5 min then weighed. The cooking water was collected then dried in an air oven at 105 ℃ to a constant weight of residue. The cooking loss was expressed as the ratio of the weight of residue to the original noodle weight. The cooked weight was expressed as the ratio of the increased weight of cooked rice noodle to the original weight of the noodle on a semi-dry basis. Three tests were made on each sample.

1.5 Statistical analysis

The statistical analysis was conducted using the Statistical Package for Social Sciences (Version 19.0; SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was used to investigate the differences in technological traits between the isolates. The results of enzyme activity, growth value, and acidification ability were converted into four qualitative codes, 0, 1, 2, and 3 (Table 1), then used for the multivariate analysis. The data on the quality of the rice noodles, expressed as mean±standard deviation, were analyzed using ANOVA. The Duncan test was used for investigating differences between means, being deemed significant where $P<0.05$.

表 1 主成分分析定性参数†

Table 1 Qualitative codes used for principal component analysis

Enzyme activity[a]	Growth ability[b]	Acidification capability[c]	Qualitative codes
−	$GA<0.1$	$r<0.03$	0
+	$0.1<GA<0.4$	$0.03<r<0.04$	1
++	$0.4<GA<0.7$	$0.04<r<0.05$	2
/	$GA>0.7$	$r>0.05$	3

† a. Catalase, β-glucosidase, lipase, α-amylase, and proteolytic enzyme activities. b. Growth ability at various pH values and temperatures. c. Acidification capability in sterile rice extract (SRE).

2 Results

2.1 Technology characterization

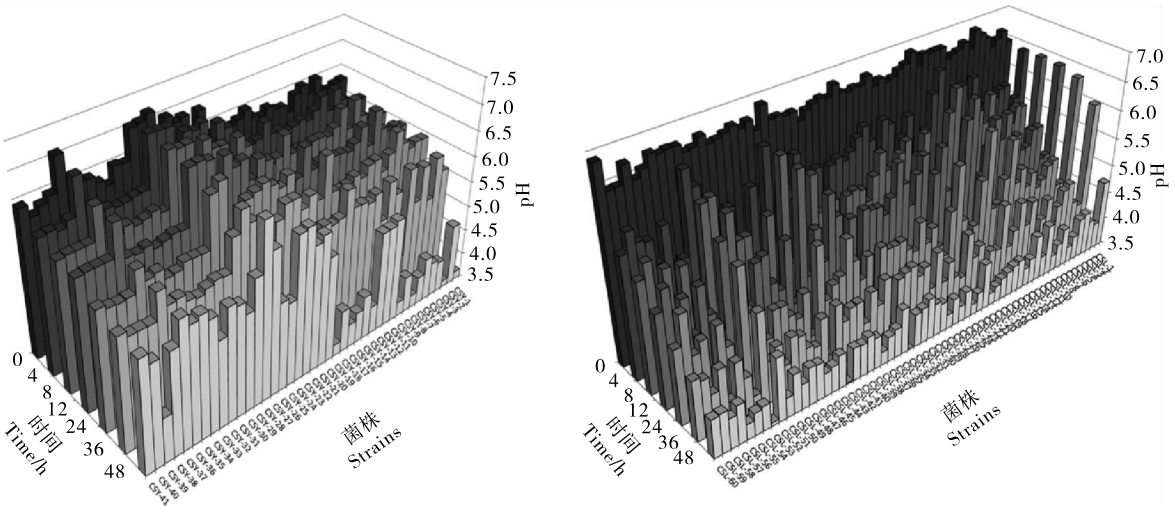
Overall, 41 yeast and 60 LAB isolates were isolated from the spontaneous fermentation of rice. The technological characteristics of these isolates were then tested.

Of the 41 yeast isolates (Table 2), 40 possessed catalase activity, with activity being strong (++) in 36 isolates and weak (+) in 4 isolates. β-glucosidase activity was detected in 12 isolates: 10 isolates with weak and 2 with strong (CSY 10 and 17) activity. Of the remainder, 22 isolates exhibited lipase activity (17 with weak and 5 with strong activity), 10 isolates showed α-amylase activity (9 with weak and 1 with strong activity), and 9 isolates showed proteolytic activity (4 with weak and 5 with strong activity). The growth ability of the isolates was defined by their growth value. An incubation temperature of 10 ℃ could inhibit yeast growth, indicated by *GA* values below 0.1 for CSY 38 and 41. However, there was no significant difference between the growth of yeast isolates at 25, 40 ℃. Regarding pH, growth was generally inhibited at pH 3 and 7, but eight isolates (CSY 01, 07, 13, 14, 15, 27, 30, and 40) showed high *GA* values at pH 3, 4, 5, 6, and 7. Only 2 isolates (CSY 07 and 19) reduced the pH of SRE below 5.0 after 36 h. After 48 h, 6 isolates (CSY 01, 03, 08, 10, 17, and 19) acidified the broth to a pH below 4.0.

Finally, the technological traits of the yeast isolates were analyzed using PCA. A biplot of principal component 1 (variability of 21.6%) and principal component 2 (variability of 16.3%) was constructed [Figure 1(a)]. Two important groups, A1 and A2, could be distinguished according to the position of the variables on the PCA map. The group A1 included the isolates CSY 01, 13, 15, and 40, showing high growth at pH 3, 5, and 6; the group A2 included the isolates CSY 07, 14, 27, and 30 showing high growth at pH 4 and 7. Apart from these two groups, some interesting isolates were observed (Table 2): CSY 16 had the trait of strong lipase activity, CSY 17 the traits of strong α-amylase, proteolytic, and β-glucosidase activities, and CSY 41 the trait of strong proteolytic enzyme activity. Therefore, the isolates, CSY 01, 07, 13, 14, 15, 16, 17, 27, 30, 40, and 41, were selected for the next phase of analysis.

The results of the technological characterization of LAB are shown in Table 3. Regarding extracellular enzymes, 23 isolates exhibited no catalase activity, with activity being strong (++) for 30 isolates and weak (+) for 7 isolates. Proteolytic and α-amylase activities were only detected in 3 isolates (CSL 13, 29, and 31); β-glucosidase activity was detected in 13 isolates (3 with weak and 10 with strong

activity, with the strongest values exhibited by isolates CSL 06 and 23). Otherwise, 36 isolates possessed lipase activity (25 with weak and 11 with strong activity). Regarding growth ability at different temperatures, 31 isolates showed strong growth ability ($GA > 0.7$) at 40 ℃ and 10 isolates at 25 ℃. At 10 ℃, all isolates exhibited low growth ability ($GA < 0.4$). Regarding the effect of pH, 49 isolates showed high growth at pH 4, 5, 6, and 7 but at pH 3, the growth of all isolates was inhibited. The SRE pH fell below 5.0 in 8 isolates after 4 h and in 21 isolates after 12 h. After 48 h, all isolates had acidified the broth to a pH below 4.0 and 31 isolates to a pH below 3.0.



(a) 酵母菌　　(b) 乳酸菌

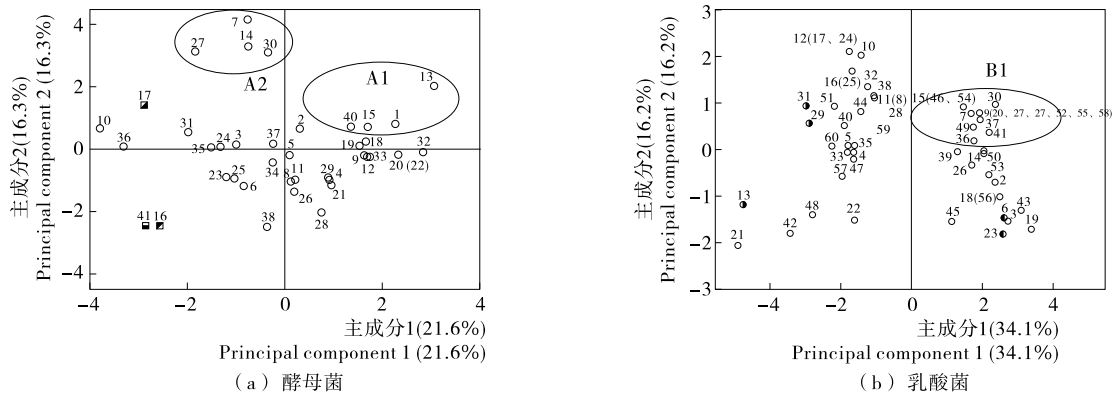
图 1　酵母菌和乳酸菌在大米提取物培养基(SRE)中的培养时的 pH 值变化

Figure 1　Kinetics of acidification in sterile rice extracts (SRE) of isolated yeasts and lactic acid bacteria

The technological traits of the LAB isolates were also analyzed using PCA. A biplot of principal component 1 (variability of 34.1%) and principal component 2 (variability of 16.2%) was constructed[Figure 1(b)]. According to the position of the variables on the PCA map, the B1 group, consisting of CSL 07, 09, 15, 20, 27, 30, 36, 37, 41, 46, 49, 54, 55, and 58, was distinguished by showing a strong growth ability at pH values of 3, 4, 5, 6, and 7 and temperatures of 10, 25, and 40 ℃. Other interesting isolates were also identified: CSL 13, 29, and 31 possessed strong α-amylase and proteolytic enzyme activities and CSL 06 and 23 had strong lipase and β-glucosidase activities. Thus, these technologically relevant isolates, CSL 06, 07, 09, 13, 15, 20, 23, 27, 29, 30, 31, 36, 37, 41, 46, 49, 54, 55, and 58, were selected for further analysis.

2.2　Genotypic identification

The genotype and phylogenetic trees of the technologically relevant isolates were identified and are shown in Figure 2(a) and Figure 2(b). For yeasts, the isolates, CSY 01, 15, 17, and 40, were classified as *Lachancea* spp with one isolate, CSY 13, identified as *S. cerevisiae*. Otherwise, the isolates CSY 07, 14, 16, 27, 30, and 41, showed a high degree of similarity and could be classified as *Trichosporon* spp.



(a) 酵母菌　　(b) 乳酸菌

图 2　酵母菌和乳酸菌发酵性能主成分分析图

Figure 2　Principal component analysis (PCA) of the technological traits of yeasts and lactic acid bacteria

表 2 自然发酵大米分离的酵母菌发酵性能参数†

Table 2 Technological characteristics of the yeast isolates from spontaneously fermented rice

Isolates	Activity of different enzymes[a]					Growth at different temperatures[b]			Growth at different pH values[c]					Acidification capability (r)[d]
	C	β-G	L	A	P	10 ℃	25 ℃	40 ℃	3	4	5	6	7	
CSY01	++	−	++	−	−	0.1～0.4	>0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	0.04～0.05
CSY02	++	+	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	0.4～0.7	<0.03
CSY03	++	+	+	−	+	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.04～0.05
CSY04	++	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	0.4～0.7	0.03～0.04
CSY05	++	−	++	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.05
CSY06	+	−	−	−	−	0.1～0.4	0.4～0.7	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY07	++	+	+	−	+	0.1～0.4	>0.7	0.1～0.4	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	>0.05
CSY08	++	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.04～0.05
CSY09	++	−	+	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	0.03～0.04
CSY10	+	++	+	−	−	0.1～0.4	0.4～0.7	0.1～0.4	0.1～0.4	0.4～0.7	0.1～0.4	0.4～0.7	0.4～0.7	0.04～0.05
CSY11	++	−	+	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	0.4～0.7	0.4～0.7	<0.03
CSY12	++	−	−	+	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	<0.03
CSY13	++	−	+	−	−	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	<0.03
CSY14	++	+	+	+	+	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	>0.7	0.4～0.7	>0.7	>0.7	0.03～0.04
CSY15	++	−	+	++	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	0.03～0.04
CSY16	++	+	++	−	−	0.1～0.4	0.4～0.7	>0.7	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.03～0.04
CSY17	++	++	−	+	++	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.03～0.04
CSY18	++	−	+	+	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	0.03～0.04
CSY19	++	−	+	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	>0.05
CSY20	++	−	−	−	−	0.1～0.4	>0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	<0.03
CSY21	++	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	0.4～0.7	<0.03
CSY22	++	−	−	−	−	0.1～0.4	>0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	<0.03
CSY23	+	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY24	++	+	+	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY25	++	−	+	−	−	0.1～0.4	0.1～0.4	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.04～0.05
CSY26	++	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY27	++	+	++	+	++	0.1～0.4	0.4～0.7	0.1～0.4	0.1～0.4	>0.7	>0.7	0.4～0.7	0.4～0.7	<0.03
CSY28	++	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	0.4～0.7	0.1～0.4	<0.03
CSY29	++	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	0.4～0.7	0.4～0.7	<0.03
CSY30	++	+	+	+	−	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	<0.03
CSY31	++	+	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	>0.7	0.4～0.7	0.1～0.4	>0.7	<0.03
CSY32	++	−	+	−	−	0.1～0.4	>0.7	>0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	<0.03
CSY33	++	−	−	+	+	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	0.4～0.7	<0.03
CSY34	+	−	−	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY35	++	−	+	−	++	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY36	−	+	−	−	++	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY37	+	−	+	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	>0.7	<0.03
CSY38	++	−	−	−	−	<0.1	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	<0.03
CSY39	++	−	+	−	−	0.1～0.4	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.03～0.04
CSY40	++	−	++	+	−	0.1～0.4	0.4～0.7	>0.7	0.1～0.4	0.4～0.7	>0.7	>0.7	0.4～0.7	<0.03
CSY41	++	−	−	+	++	<0.1	0.4～0.7	0.4～0.7	0.1～0.4	0.1～0.4	0.1～0.4	0.4～0.7	0.4～0.7	<0.03

† a. Catalase (C), β-glucosidase (β-G), lipase (L), α-amylase (A) and proteolytic enzyme (P) activities; b. Growth of yeasts incubated at different pH values; c. Growth of yeasts incubated at different temperatures; d. Acidification capability of yeasts in sterile rice extract (SRE).

表 3　自然发酵大米分离的乳酸菌发酵性能参数†

Table 3　Technological characteristics of LAB isolates from spontaneously fermented rice

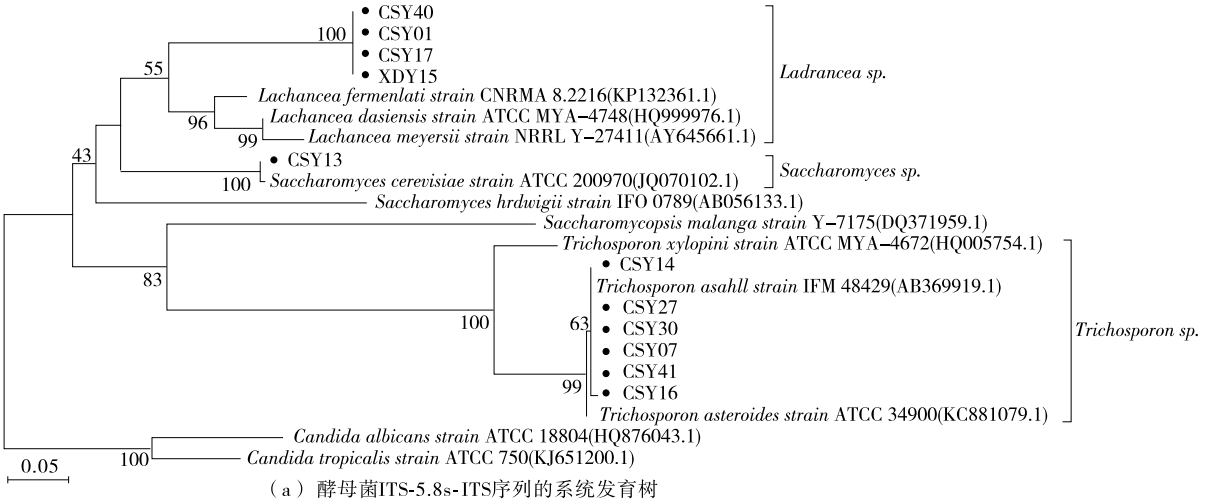
Isolates	Enzymes[a]					Temperature[b]			pH[c]					Acidification capability (r)[d]
	C	β-G	L	A	P	10 ℃	25 ℃	40 ℃	3	4	5	6	7	
CSL01	++	−	+	−	−	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.03～0.04
CSL02	+	+	++	−	−	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL03	−	++	++	−	−	0.1～0.4	>0.7	0.1～0.4	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL04	++	−	+	−	−	0.1～0.4	0.1～0.4	0.1～0.4	<0.01	>0.7	>0.7	0.4～0.7	0.4～0.7	0.04～0.05
CSL05	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	0.4～0.7	0.4～0.7	>0.7	0.04～0.05
CSL06	−	++	++	−	−	0.1～0.4	>0.7	>0.7	0.1～0.4	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL07	−	−	+	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	>0.05
CSL08	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL09	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL10	++	−	−	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	>0.7	>0.7	>0.05
CSL11	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	0.04～0.05
CSL12	++	−	−	−	−	<0.1	<0.1	<0.1	0.1～0.4	>0.7	>0.7	>0.7	>0.7	>0.05
CSL13	++	−	+	++	++	<0.1	0.1～0.4	<0.1	<0.1	0.4～0.7	0.4～0.7	>0.7	0.1～0.4	0.04～0.05
CSL14	−	−	++	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL15	+	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL16	++	−	+	−	−	<0.1	<0.1	<0.1	0.1～0.4	>0.7	>0.7	>0.7	>0.7	>0.05
CSL17	++	−	−	−	−	<0.1	<0.1	<0.1	0.1～0.4	>0.7	>0.7	>0.7	>0.7	>0.05
CSL18	−	++	++	−	−	<0.1	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL19	−	++	++	−	−	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL20	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL21	++	−	−	−	−	<0.1	<0.1	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	>0.05
CSL22	++	−	+	−	−	0.1～0.4	0.1～0.4	0.1～0.4	<0.1	0.4～0.7	0.4～0.7	0.4～0.7	0.4～0.7	0.03～0.04
CSL23	−	++	++	−	−	0.1～0.4	>0.7	>0.7	0.1～0.4	>0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL24	++	−	−	−	−	<0.1	<0.1	<0.1	0.1～0.4	>0.7	>0.7	>0.7	>0.7	>0.05
CSL25	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	>0.7	>0.7	>0.05
CSL26	−	−	+	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL27	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL28	++	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.1～0.4	>0.7	>0.7	0.1～0.4	>0.7	>0.05
CSL29	++	−	+	++	++	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL30	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	>0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL31	++	−	+	++	++	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	0.04～0.05
CSL32	++	−	+	−	−	<0.1	<0.1	0.4～0.7	0.1～0.4	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL33	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	<0.1	>0.7	0.4～0.7	>0.7	0.4～0.7	0.04～0.05
CSL34	+	++	++	−	−	0.1～0.4	>0.7	>0.7	<0.1	<0.1	<0.1	>0.7	0.1～0.4	0.04～0.05
CSL35	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	<0.1	>0.7	>0.7	>0.7	0.4～0.7	0.03～0.04
CSL36	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.1～0.4	>0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL37	+	−	−	−	−	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL38	++	+	+	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	>0.7	>0.7	>0.05
CSL39	−	−	−	−	−	<0.1	0.4～0.7	>0.7	0.4～0.7	0.4～0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL40	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	<0.1	>0.7	>0.7	>0.7	0.4～0.7	0.04～0.05
CSL41	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL42	++	−	−	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	0.1～0.4	>0.05
CSL43	−	++	++	−	−	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL44	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	0.4～0.7	>0.7	0.04～0.05
CSL45	−	++	−	−	−	<0.1	>0.7	0.4～0.7	<0.01	0.4～0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL46	+	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL47	++	−	+	−	−	<0.1	<0.1	0.1～0.4	0.1～0.4	0.1～0.4	>0.7	>0.7	>0.7	0.04～0.05
CSL48	++	−	+	−	−	<0.1	0.1～0.4	<0.1	0.1～0.4	0.4～0.7	0.4～0.7	0.1～0.4	0.4～0.7	0.04～0.05
CSL49	+	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.03～0.04
CSL50	−	++	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05

续表 3

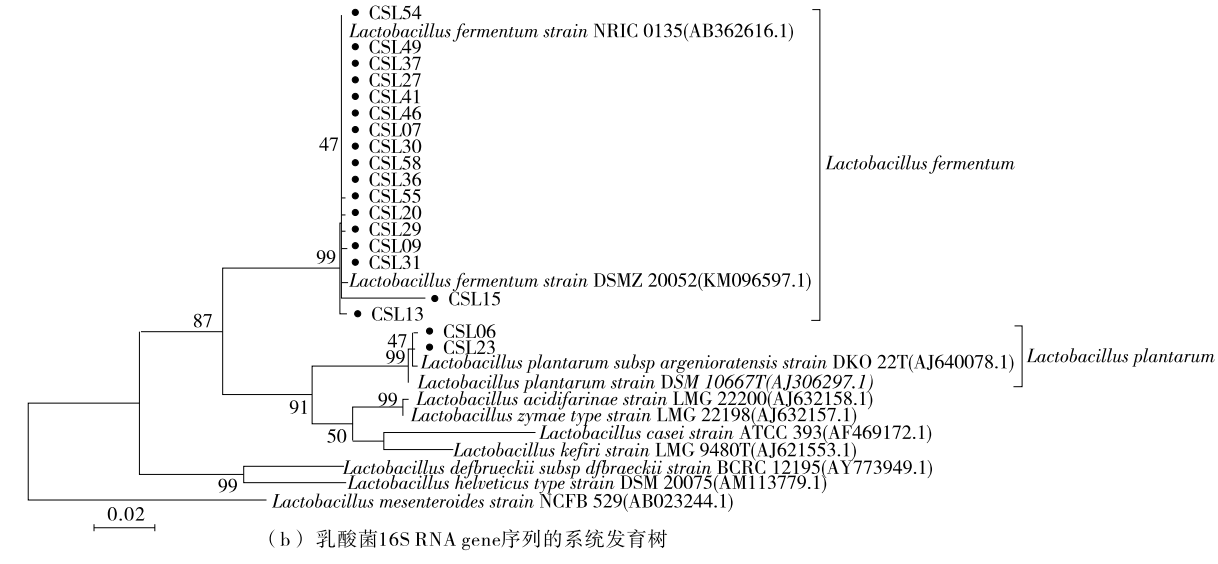
Isolates	Enzymes[a]					Temperature[b]			pH[c]					Acidification capability (r)[d]
	C	β-G	L	A	P	10 ℃	25 ℃	40 ℃	3	4	5	6	7	
CSL51	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	<0.1	>0.7	0.4～0.7	>0.7	0.4～0.7	>0.05
CSL52	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL53	−	+	++	−	−	<0.1	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL54	+	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL55	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL56	−	++	++	−	−	<0.1	>0.7	>0.7	0.4～0.7	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL57	++	−	+	−	−	<0.1	0.1～0.4	>0.7	<0.1	>0.7	<0.1	>0.7	<0.1	0.04～0.05
CSL58	−	−	−	−	−	0.1～0.4	0.4～0.7	>0.7	0.1～0.4	>0.7	>0.7	>0.7	>0.7	0.04～0.05
CSL59	++	−	+	−	−	<0.1	0.1～0.4	>0.7	0.1～0.4	>0.7	0.4～0.7	>0.7	>0.7	0.04～0.05
CSL60	++	−	+	−	−	<0.1	0.1～0.4	0.1～0.4	<0.1	>0.7	>0.7	>0.7	0.1～0.4	0.04～0.05

† a. Catalase (C), β-glucosidase (β-G), lipase (L), α-amylase (A) and proteolytic enzyme (P) activities; b. Growth of lactic acid bacteria incubated at different pH values; c. Growth of lactic acid bacteria incubated at different temperatures; d. Acidification capability of lactic acid bacteria in sterile rice extract (SRE).

Regarding LAB(Figure 3), two species could be classified: the isolates CSL 06 and 23 were identified as from the *L. plantarum* group and the isolates CSL 07, 09, 13, 15, 20, 27, 29, 30, 31, 36, 37, 41, 46, 49, 54, 55, and 58 as *L. fermentum*, but CSL 13 and 15 were a relatively long evolutionary distance from the others.



(a) 酵母菌ITS-5.8s-ITS序列的系统发育树



(b) 乳酸菌16S RNA gene序列的系统发育树

图 3 采用最大似然法(ML)构建的酵母菌 ITS-5.8s-ITS 序列的系统发育树和乳酸菌 16S RNA gene 序列的系统发育树

Figure 3 Phylogenetic tree based on the ITS-5.8s-ITS sequences of isolated yeasts and the complete 16S rRNA gene sequences of isolated LAB using the maximum likelihood (ML) method

Thus, isolates with interesting technological traits and antimicrobial activity were selected: CSY 01 possessed a strong acidification capability compared with the others from the *Lachancea* spp. group; CSY 07 had stronger extracellular enzymes activity, higher growth at different pH values and temperatures, and a stronger acidification capacity than others from the *Trichosporon* spp. group; in the *L. fermentum* group, CSL 30 showed higher quality technological traits and more significant antimicrobial activities, but CSL 13 and 15 may be subspecies, and may thus be selected for further tests as particular isolates; CSL 23 possessed a stronger antimicrobial activity than CSL 06 in *Lactobacillus plantarum*. Thus, the isolates CSY 01, 07, and 13 as well as CSL 13, 15, 23, and 30, were selected for fermenting the rice noodles.

2.3 Quality of rice noodles

The textural and cooking qualities of rice noodles fermented using the different isolates were significantly different ($P<0.05$) (Table 4). The tensile strength of cooked rice noodles fermented by CSL 23 and 30 was significantly higher than those fermented by the other isolates. The hardness of noodles fermented by CSL 15, 23, and 30 was significantly higher than that of other samples. The cooked noodles fermented by CSL 15 and 23 exhibited lower adhesiveness values than the naturally fermented noodles. The noodles fermented by the yeast isolates (CSY 01, 07, and 13) were less cohesive than the naturally fermented noodles, which were less cohesive than noodles fermented by the LAB isolates. Regarding cooking quality, cooking loss was generally negatively correlated with cooked weight. The noodles fermented by CSY 13 and CLS 23 and 30 had significantly lower cooking losses and higher cooked weight than noodles fermented using the other isolates.

表 4　不同菌种发酵鲜湿米粉的质构和蒸煮品质†

Table 4　Textural and cooking quality of rice noodles fermented with different starter cultures

Starter culture	Tensile Strength/kg	Hardness/kg	Adhesiveness/(g·s)	Cohesiveness	Cooking Loss/%	Cooked Weight/%
None[n]	20.75 ± 1.10^{ab}	0.168 ± 0.013^{ab}	25.92 ± 1.34^{a}	0.69 ± 0.28^{abcd}	4.84 ± 0.49^{abc}	97.93 ± 2.14^{ab}
CSY01	19.92 ± 0.35^{a}	0.145 ± 0.006^{a}	25.64 ± 1.44^{a}	0.68 ± 0.31^{abc}	4.68 ± 0.56^{abc}	108.27 ± 5.74^{c}
CSY07	19.26 ± 0.66^{a}	0.145 ± 0.010^{a}	37.63 ± 5.42^{b}	0.67 ± 0.32^{ab}	5.68 ± 0.07^{c}	99.75 ± 5.72^{ab}
CSY13	23.38 ± 0.77^{ab}	0.173 ± 0.014^{abc}	25.14 ± 1.06^{a}	0.64 ± 0.18^{a}	3.35 ± 0.59^{a}	106.41 ± 3.20^{ab}
CSL13	20.85 ± 2.18^{ab}	0.160 ± 0.007^{a}	27.48 ± 0.94^{a}	0.75 ± 0.11^{cde}	4.65 ± 0.20^{abc}	109.01 ± 6.16^{c}
CSL15	22.74 ± 1.46^{ab}	0.198 ± 0.012^{bc}	24.45 ± 1.32^{a}	0.75 ± 0.14^{bcde}	5.48 ± 0.19^{bc}	94.00 ± 3.19^{a}
CSL23	24.87 ± 0.83^{c}	0.204 ± 0.002^{c}	22.35 ± 4.48^{a}	0.76 ± 0.28^{de}	3.87 ± 0.82^{ab}	109.99 ± 1.28^{c}
CSL30	24.68 ± 0.20^{c}	0.197 ± 0.009^{bc}	30.16 ± 2.93^{ab}	0.78 ± 0.27^{be}	3.97 ± 0.95^{ab}	108.85 ± 0.76^{c}

† Mean values in the same column bearing superscripts with the same letters are not significantly different ($P>0.05$); n. Natural fermentation without starter culture.

3 Discussion

A promising strain of microorganism for starter cultures in rice noodle manufacturing must have some desirable traits: functional extracellular enzyme activity, high growth over a wide range of pH values and temperatures, a strong acidification capability and a strong antimicrobial ability.

Extracellular enzymatic activity is a critical property for a strain to be used as a starter because it can modify the quality of raw material through producing some metabolites and secondary compounds (Bevilacqua, Corbo, & Sinigaglia, 2012). β-glucosidase can help release secondary compounds which can potentially enhance the flavor of foods (Palmeri & Spagna 2007). Catalase can prevent the oxidation reaction to help improve the quality of the food material (Bevilacqua, Perricone, Cannarsi, Corbo, & Sinigaglia, 2009). Previous studies have shown that changes in rice granules during the fermentation process, such as slight etching of the starch crystal and reduced contents of lipid and protein, can improve the quality of rice noodles (Lu, Li, Min, Wang, & Tatsumi, 2005). Thus, it can be concluded that α-amylase, lipase, and proteolytic enzymes were active in the isolates examined in the present study.

To evaluate the effectiveness of a starter at different pH values and temperatures, the growth ability of isolates was calculated at incubation temperatures of 10, 25, and 40 ℃ and at pH values of 3, 4, 5, 6, and 7. From an industrial point of view, starters with high growth ability under ordinary conditions are easy to culture in large quantities (Bevilacqua, Beneduce, Sinigaglia, & Corbo, 2013). To be relevant to industrial conditions, the assay of growth ability in the present study was conducted under aerobic conditions.

As the results detailing the technological characteristics are multifactorial, selecting the most promising isolates cannot be achieved through simple analysis techniques. Principal component analysis can be used to reduce the dimensionality of the data. Based on this multivariate approach, as detailed earlier, 11 yeast isolates and 19 LAB isolates were selected as candidates for preparing improved starters.

Determining the sequence of the 16S rRNA gene is an important technique for differentiating prokaryotic microor-

ganisms (Nagpal, Fox, & Fox, 1998). For the fungal kingdom, the ITS sequence has generally been applied for species discrimination and has a more clearly defined barcode gap for fungi (Schoch, Seifert, Huhndorf, Robert, Spouge, Levesque, & Chen, 2012). Based on these methods, the technologically relevant isolates were identified and classified. By combining the technological traits with antimicrobial activity, the promising species were selected for producing fermented rice noodles.

Rice noodles of high quality require several special textural and cooking traits: a higher tensile strength, hardness, cohesiveness, and cooked weight, and a lower adhesiveness and cooking loss. Tensile testing assesses the breaking strength of noodles to act as an indicator of their quality (Fari, Rajapaksa, & Ranaweera, 2011). Hardness has been shown to be a dominant factor in the quality perception of rice noodles, being negatively correlated with cooking loss, but positively correlated with cooking weight (Wang, Warkentin, Vandenberg, & Bing, 2014). As rice noodles have low adhesiveness, they are easy to separate from each other. This lower adhesiveness as well as high cohesiveness can improve aspects of mouthfeel, such as increasing the slipperiness of the noodles (Chen, Sagis, Legger, Linssen, Schols, & Voragen, 2002). In the present study, rice noodles fermented by the LAB isolate, CSL 23, exhibited the required qualities of higher tensile strength, higher hardness, lower adhesiveness, higher cohesiveness, lower cooking loss, and higher cooked weight.

In the ecological system of fermented foods, the actions of yeasts and LAB cannot be completely separated, as they can interact through molecular, nutritional, and metabolic factors (Frey-Klett, Burlinson, Deveau, Barret, Tarkka, & Sarniguet, 2011). These symbiotic interactions include mutualism, commensalism, and parasitism. Stadie, Anna, Ehrmann, & Vogel (2013) have described the interaction between yeasts and LAB in a water/kefir medium and demonstrated that they both significantly increased cell yield in a co-culture system because of molecular interaction, trophic interaction, and metabolite exchange. Tada, Katakura, Ninomiya, & Shioya (2007) used the symbiotic interactions between *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae* to enhance the production of kefiran. In the present study, rice noodles fermented with the yeast isolate, CSY 13, exhibited better qualities of higher tensile strength, higher hardness, lower adhesiveness, and lower cooking loss than noodles fermented by the other yeast isolates.

In conclusion, two potential isolates, *Saccharomyces cerevisiae* (CSY 13) and *Lactobacillus plantarum* (CSL 23), have been shown to be the most suitable starter cultures for producing fermented rice noodles.

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信息窗

“无害”食品添加剂或“无意”导致流行病出现

英国《自然》杂志近日发表的一篇微生物学论文报告称,美国科学家通过全基因组测序和对比后认为,艰难梭菌(Clostridium difficile)的高毒菌株获得了代谢海藻糖的机制,很可能正是一种广泛使用的食品添加剂,“无意”中导致了这些流行菌株的出现。

艰难梭菌是一种肠道病原菌,如果人体或动物过度服用某些抗生素,就会导致艰难梭菌菌群生长速度过快,影响肠道中其它细菌,进而引发炎症,所以这种菌被视为抗生素相关腹泻的主要原因。

美国贝勒医学院研究人员罗伯特·布雷顿及其同事,通过全基因组测序和对比分析发现,两种在系统发生学上存在显著差异的艰难梭菌高毒流行核糖体分型——RT027和RT078,这两种核糖体分型不断变异,使艰难梭菌在低浓度海藻糖中的生长能力逐渐增强。更重要的是,这种能力与人化小鼠模型的疾病严重程度相关。

数据揭示,该核糖体分型的出现与一种糖添加剂有关,该添加剂广泛应用于人类饮食中的海藻糖。研究人员表示,这之间的关联可表明,一种本身“无害”的食品添加剂,也可能“无意”中促进了病原菌的出现。

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