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Selection of the Optimal Association Between Lactic Acid Bacteria and Yeasts in Chinese Sourdoughs

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The aim of this work was to study the interactions between LAB and yeasts isolated from five traditional Chinese sourdoughs. The characteristic of five sourdoughs were analyzed. Dough fermentation ability, pH, TTA, lactic acid and acetic acid were determined for selection the yeast strains and LABs were isolated from these sourdoughs. They were identified as *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei*. After choosing three interesting strains for each kind of microorganism, they were studied individually or combined and compared to the sourdough.

Key words: Chinese sourdough; LAB; yeasts; interaction.

Sourdough fermentation is one of the oldest cereal fermentations in mankind[1]. The most frequently encountered genera of sourdough LAB is hetero-fermentative *Lactobacillus*, responsible for souring the dough, secreting aroma compounds or aroma precursors, producing polysaccharide and antifungal molecules[2]. Yeasts, dominant species *Saccharomyces cerevisiae*, metabolize fermentable sugars producing CO_2 as a leavening agent and enhance volume[3]. The interactions between these microorganisms can influence the shelf-life, the rheological and organoleptic (by producing aromas) properties of the dough[4,5]. They greatly influence the properties of the sourdough and eventually, the quality of the final product.

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The aim of this study was to provide more information about the effect of different associations between yeasts and LAB during sourdough fermentation. To do it, we first characterized 5 sourdoughs and then worked on the yeasts and bacteria isolated from them.

MATERIALS AND METHODS

Collection and chemical analysis of sourdough samples

Five Chinese sourdoughs were aseptically collected from China. They were Hn from a steamed bread shop in Henan province, Sx from a friend's home in Shanxi province, Gs from a friend's home in Gansu province, Hf from steamed bread shop in Anhui province and Hr from a friend's home in Heilongjiang province . The pH value of sample was determined by standard methods[6]. Total titratable acidity (TTA) was measured on 10 g of sourdough samples, homogenized with 90 ml of distilled water for 1 min and expressed as the

amount of 0.1M NaOH (ml) to achieve the pH 8.3.

Isolation of yeasts and LABs

Ten grams of sourdough was homogenized with 90 ml sterile water solution, a tenfold serial dilution in sterile water solution was carried out. For yeasts, 0.1 ml each serial dilution (10^{-1}-10^{-6}) were added into Yeast extract-Peptone-Dextrose (PDA) agar with 150 ppm chloramphenicol for counting, and incubated at 28 °C for 5 d. For LABs, 0.1 ml each serial dilution (10^{-1}-10^{-9}), aliquots in were added into Man-Rogosa-Sharpe (MRS), incubated at 37 °C for 48 h.

Selection yeasts and LABs

For selection yeasts, the ability to increase the volume of the dough of yeast isolated was studied. 200g of dough with yeast were put in a 1L graduated cylinder with 40 mL of paraffin to read the volume. The leavening was calculated as: $[(A_1–A_0) / A_0] \times 100]$, where A_0 is the initial volume and A_1 is the volume after the 4 hours of fermentation at 30°C and 75% relative humidity . For LABs, pH, TTA and lactic acid and acetic acid of LABs isolated were studied.

Identification isolated strains

DNA extraction was performed according to manufacturer's instructions of the DNA extraction kit. 50 µl PCR solution containing : 1×PCR buffer, 200 µM dNTP, 0.4 µM each primer (27f (5'-AGRGTTTGATYVTGGCTCAG-3') and 1492r (5'-TACGGHTACCTTGTTACGACTT-3') for LABs and NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and RLR3R (5'-ATTCCCAAACAACTCGACTC-3') for yeasts), 1.25 U of Ex Taq polymerase and 2 µl of extracted DNA. PCR conditions were: 95 °C for 4 min, 30 cycles of 95 °C for 30 s, annealing temperature (58 °Cfor LABs and 55 °C for yeasts) for 30 s, 72 °C for 1 min, and finally 72 °C for 10 min. The PCR products were sequenced by Shanghai Sangni Biosciences Corporation and sequence homologies were examined with NCBI database (http://blast.ncbi.nlm.nih.gov/Blast).

Dough fermentation with selected strains

The dough was made with 450g of wheat flour, 225g of water and starters or 85 g of sourdough. The LABs and yeasts broth cultures were centrifuged at 3000 rpm for 10 min, and resuspended in distilled water. The cell suspensions were diluted to an optical density of 1.25 at 620 nm (OD_{620})[7]. Dough was incubated for 4 hours at 30°C and 75% humidity. The control group was non-fermented dough with only flour and water.

Determination of cell numbers and organic acid

Cell counts (expressed as cfu/g of dough) were determined by mixing 5 g of fresh sourdough with 45 mL of peptone physiological solution 0.1% of bacteriological peptone and 0.85% of NaCl.Organic acid were determined on the supernatant described by GB/T GB/T 5009.157-2003, determination of organic acid in foods[8].

Statistical analysis

All experiments were performed in triplicate. Results were statistic by SPSS 17.0.

RESULTS AND DISCUSSION

Characterization of the five sourdoughs

Characterization of five Chinese sourdough were shown in Table 1.

The amounts of LAB ranged from 7.20 to 8.01 Log cfu/g sample, while the yeast counts ranged from 5.23 to 7.21 Log cfu/g sample. The pH values of all samples were in agreement with their respective TTA results , indicating the data of pH represented the total acidity of the sourdough samples.

Selection of the yeast and LABs strains

Sx2, Gs1 and Hr1 strains were the higher increase in the volume of the dough with values of 15.25%, 13.52% and 19.44% respectively (Fig.1). The values for the other strains are all below *10%. They were identified as* Saccharomyces cerevisiae.

The TTA values give an indication on the total acidity found in the sourdough whereas the pH only indicates the amount of strong acid produced. In Table 2, LABs of Sx7, Gs3 and Hr 22 were the lower pH value (3.68, 4.25 and 4.05) and higher TTA value (6.17,4.13 and 5.77) than others. They also produced more lactic acid and acetic acid (Table 2). They were identified as *L.plantarum* (Sx7), *L. casei* (Gs3) and *L. plantarum* (Hr 22) (Fig.2).

Study of the single and mixed starters

Initial and final yeast and LAB cells counts were performed on all kind of doughs. The microorganisms were inoculated between 10^5 and 10^6 for yeasts and between 10^7 and 10^8 for the LAB. After 4 hours, each species grew of approximately 1 log.The results for the mixed strains were

Table 1. Features of Chinese sourdoughs in this study

Sample	pH[a] value	TTA[a] (ml)	Log cfu/g sample[a]	
			LAB	Yeasts
Hr	4.89±0.03	10.64±0.09	7.26±0.54	6.70±0.36
Gs	4.13±0.07	11.40±0.05	7.34±0.65	6.78±1.12
Hf	5.51±0.02	7.57±0.05	7.20±0.53	5.91±0.60
Sx	3.76±0.86	13.76±0.60	7.54±0.78	5.23±0.42
Hn	5.02±0.92	9.74±0.06	8.01±0.24	7.21±0.42

[a]Data represent as mean ±SD.

Table 2. pH and TTA values for the 8 LAB strains and the 3 selected LAB and Yeast strains inoculated single or mixed

Strains	pH[a] value	TTA[a] (ml)	Lactic acid[a]	Acetic acid[a]
Sx7	3.68±0.03	6.17±0.54	2.06±0.13	0.32±0.24
Hf8	5.03±0.11	3.07±0.14	0.81±0.11	0.05±0.54
Gs3	4.25±0.42	4.13±0.17	1.86±0.22	0.23±0.47
Hn15	4.71±0.13	3.40±0.33	1.11±0.53	0.08±0.14
Hr22	4.05±0.34	5.77±0.06	1.98±0.34	0.27±019
Hf9	5.22±0.54	2.37±0.03	0.62±0.04	0.07±0.16
Hr23	5.51±0.21	2.63±0.09	0.51±0.31	0.03±0.49
Sx2	5.60±0.22	2.00±0.07	0.60±0.12	0.10±0.16
Control dough	5.67±0.11	2.87±0.23	-	-
S. cerevisiae Sx2	5.45±0.13	3.13±0.12	-	-
S. cerevisiae Hr1	5.41±0.11	3.27±0.17	-	-
S. cerevisiae Gs1	5.50±0.03	2.57±0.31	-	-
S. cerevisiae Sx2 + *L.plantarum* Sx7	4.26±0.13	5.03±0.10	1.26±0.10	0.15±0.25
S. cerevisiae Hr1 + *L.plantarum* Hr22	4.73±0.32	4.93±0.15	1.23±0.21	0.13±0.13
S. cerevisiae Gs1 + *L. casei* Gs3	4.49±0.09	4.40±0.14	0.69±0.17	0.45±0.19

[a]Data represent as mean ±SD.

compared to those of the single strains but also to those of the initial sourdoughs and are presented in Table 2. The pH of the dough made only with yeasts did not decrease a lot, contrary to the ones of doughs made with the bacteria. But the TTA values for yeast did show a small increase. Indeed, Hr1 and Sx2 are the two strains which produce the more CO_2 and their TTA values are higher than the value recorded for Gs1. For Gs3, the pH and TTA values are not significantly different from the values obtained after combining it with Gs1. When the yeasts and LAB strains were combined, the TTA values were above the ones of each strain taken individually. But the pH values are not as low as for doughs made only with LAB. The bacteria which are often found as dominant in sourdough are *L. sanfranciscensis*[9] and *L. plantarum*[10].The persistence of *L. plantarum* can be explained as its metabolism of carbohydrates has a great adaptability and is well adapted to ferment cereals[11].

Obligately or facultatively heterofermentative bacteria can also produce other metabolites such as and lactic acid and acetic acid[12]. These two acids contribute to the sourdough aroma and delay or prevent the growth of spoiling agents. It can be seen from Table 2 that the lactic acid and acetic acid were higher when single LABs in dough, however, the capacity to ferment several carbohydrates can reduce the metabolic competition with yeast and have an impact on acidification.

In this work, acidification and volume increase of doughs made with sourdough, single and mixed strains of yeasts and LAB were studied. More investigations would be necessary to be able

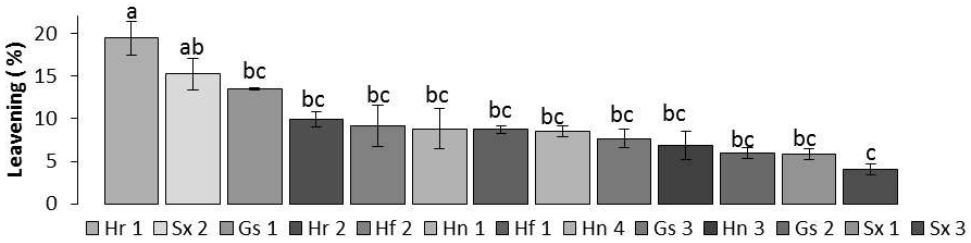

Fig. 1. Dough leavening for the isolated yeast strains. Means with different letters are significantly different (P > 0.05)

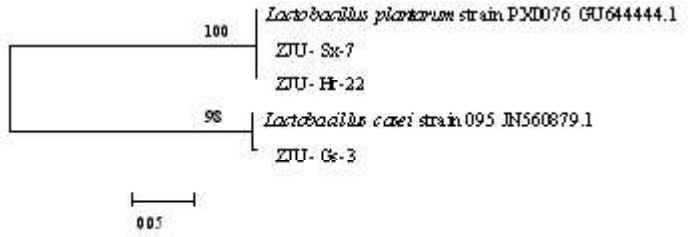

Fig. 2. Phylogenetic tree of bacteria isolates strains based on the 16S rDNA gene sequences

to characterize precisely the 5 sourdoughs studied and the interactions between their microorganisms.

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