

## Identification of Dominant Microorganisms in Pickle Juice

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**Abstract.** Using plate dilution method for 5 kinds of pickle juice in microbial identification research, the results show that lactic acid bacteria were the dominant species in the packaged, unpackaged and homemade pickles, yeast were second dominant species, *Pichia yeast* and *candida* appear in 5 kinds of pickles. The number of lactic acid bacteria were most in packaged sauerkraut termed Chaqi, mainly included in *Lactobacillus plantarum*, *Lactobacillus brevis* and *Leuconostoc*. *Lactobacillus* are dominant in Kimchi and Pickles, Sichuan Style; *Lactobacillus plantarum* and *Streptococcus lactis* in cabbage, *Lactobacillus plantarum* and *Lactobacillus brevis* in pickle contained pepper oil; *Penicillium* in the homemade pickles and the packaged sauerkraut termed Chaqi, but homemade pickles are more.

### Introduction

Pickles contains more mineral elements, dietary fiber and rich in vitamins, not only can be used as cooked food, but also is benefit for appetite, digestion and the intestinal environment, as well as promoting gastrointestinal health.

By lactic acid fermentation, alcohol fermentation and weak acetic acid fermentation, the pickles has no fat and fewer calories, a large number of carbohydrates are fermented by microorganism. At present, it has been proved by the domestic and foreign biologists that the lactic acid bacteria in the intestines are closely related to the health and longevity. With the care of health awareness, the pickles rich in lactic acid bacteria become more and more popular, but the traditional natural fermentation of pickles has been unable to meet the needs of the people. To realize the industrialized production of pickles, looking for the key microorganisms in fermentation process and screening for stain are vital to this industry. Therefore, we purify and identify the dominant microorganisms in the packaged, unpackaged and homemade pickles, in order to provide a basis for strain selection in the process of pickles, but also providing reference for the choice of high quality and safety of pickles.

### Experimental Materials and Design

#### Material Selection.

The test samples for domestic sales, people-liked pickle termed Chaqi, Kimchi, Pickles, Sichuan Style, homemade pickled cabbage and pickle contained pepper oil.

#### Experimental Design.

##### 1. Culture of Bacteria

The samples were collected in a timely manner sealed, placed in the refrigerator 4°C to save reserve. Each sample is diluted to 10<sup>-1</sup> ~ 10<sup>-7</sup>, then select three suitable gradient for counting, set 3 repetition in each gradient and a blank control, the samples were cultured by coating method and plate pouring method, PDA and beef extract peptone medium were cultured for 48 hours at 28°C pouring the prepared MRS medium for 48 hours at 37°C.

##### 2. Purification of Strains

We select the characteristic colonies, single colony obtained by streak plate method and

numbered

### 3. Identification of Strains

The strains were identified by morphological identification and characteristics reactions. Lactic acid bacteria were identified by Gram staining reaction and catalase test, as well as contact enzyme test, yeast were identified by morphological identification.

### 4. Determination of the Dominant Strains

After the culture of 48h, the plate was taken out, the number of colonies was counted by natural counting method, and the average number of colonies on the three plates with the same dilution was calculated by the following formula:

$$\text{Colony forming units per milliliter (CFU)} = \frac{\text{the same dilution three times the average number of colonies}}{\text{dilution}} * 5$$

## Results and Discussion

### Identification of Lactic Acid Bacteria.

Morphological Identification.

The colony obtained by streak plate method which termed A-J10, the results of morphology identification and arrangement of cells and Gram staining showed in Table 1

Table 1 the morphological characteristics of A-J10 strains

NO	A	B	C	D	E	F	G	H	I	J
Gram stain	G <sup>+</sup>	G <sup>-</sup>	G <sup>+</sup>	G <sup>+</sup>	G <sup>+</sup>	G <sup>-</sup>	G <sup>+</sup>	G <sup>+</sup>	G <sup>+</sup>	G <sup>+</sup>
Microscopic morphology	Rod	Oval or spherical	Short straight rod	Rod	Oval or oval	Hammer shape	Rod	Long rod	Hammer shape	Short rod, blunt ends
Arrangement mode	Single or short chain	Single irregular	Single or short chain	Single birth, paired or short chain	Single or short chain	Irregular	Single irregular arrangement	Single or pair	Random chain	Single irregular

Note: "G+" means Gram staining is positive; "G-" means Gram staining is negative.

The strain-B and strain-F of 10 strains isolated from pickle juice were gram negative, which did not conform to the characteristics of lactic acid bacteria, and the other 8 strains were gram positive bacteria, which was in line with the characteristics of lactic acid bacteria.

Biochemical Test.

The 8 strains which were gram positive bacteria were further identified by biochemical tests. The contact enzyme test was negative for the 8 strains, as shown in Table 2.

Table 2 Contact enzyme test and glucose fermentation test result of 8 strains

NO	Contact enzyme test
A	-
C	-
D	-
E	-
G	-
H	-
I	-
J	-

Note: "-" means negative.

The 8 strains were all lactic acid bacteria, of which A, D, G and H were *Lactobacillus plantarum*, as shown in Figure 1, C and J were *Lactobacillus brevis*, as shown in Figure 2, I was *Streptococcus lactis*, E was the pearl.

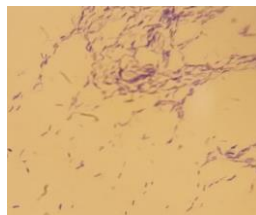


Figure 1



Figure 2

## Identification of Yeasts

### 1. Morphological Identification

The colonies which has the typical characteristics of yeast colony on PDA medium plate were purified 2 ~ 3 times and cultured 24 ~ 48 h, better strains were verified, then cultured with ring vaccination on slope (PDA), The white strain-M and white strain-N were isolated from the 5 pickle juice, and showed that colony protrusions, smooth surface, neat edge, poor transparency, easy to provoke and fermented aroma.

### 2. Microscopic Morphology

Two kinds of yeasts isolated were identified by microscopic, strain-M showed oval, oval or botuliform which diameter is 1-4mm, then *Pichia* yeast was found . The strain-N is cylindrical or irregular branching and produces pseudohypha, which found *Candida*. Fig. 3 is *Pichia* yeast, and Fig. 4 is *Candida*.

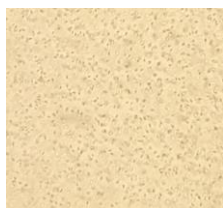


Figure 3



Figure 4

### 3. Determination of the Content of Dominant Bacteria

Based on identification of the strains, 5 kinds of microorganisms in pickles were determined. But to identify the dominant species in each kind of pickles, it is necessary to calculate the number of different strains, the results show that lactic acid bacteria is the most in in packaged sauerkraut termed Chaqi, up to  $4.8 \times 10^7$ ; yeast was the highest in homemade packle,  $3.35 \times 10^7$ ; bacteria is more,  $N \times 10^7$ , in Pickles, Sichuan Style, homemade pickle and pickled cabbage with pepper oil, but Kimchi and packaged sauerkraut termed Chaqi contain relatively few bacteria,  $N \times 10^6$ .

## Conclusion

We found that lactic acid bacteria were the highest in five kinds of pickles, 10 strains of lactic acid bacteria were isolated and purified, by morphological observation and biochemical test, 8 kinds were identified mainly including *Lactobacillus plantarum*, *Lactobacillus brevis*, *Streptococcus lactis* and *Leuconostoc* genus. The yeast is just fewer than lactic acid bacteria and can reach  $N \times 10^7$ , and *Pichia* yeast and *Candida* mainly appeared.

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