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RESEARCH ARTICLE

Effects of adding isolated *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and their mixtures on the quality indices of pickled Chinese cabbage

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ABSTRACT

Pickled Chinese cabbage traditional fermented vegetables is popular in China, especially the northeast, owing to their special flavors. Spontaneous fermented Chinese cabbage usually has rich and mellow flavor because of abundant microorganism in the fermentation broth. In our study, LAB were screened from spontaneous Chinese cabbage and identified including morphology, physiology and biochemistry, and molecular levels. 11 of these were used as a single inocula to ferment Chinese cabbage, and acid contents, soluble sugar contents, Vc contents, cellulose contents and nitrite contents of these pickled Chinese cabbage were measured. Overall, three superior strains mixture(X1, M2, and D2) were inoculated to improve the fermentation characteristics of pickled Chinese cabbage. Furthermore, pickled Chinese cabbage with the mixture had the highest acid and Vc contents, the lowest nitrite content, and medium fiber content. The sensory quality mixed inocula was also the best compared with products prepared with single strain and spontaneous fermentation.

Keywords: *Lactobacillus plantarum*; *Leuconostoc mesenteroides*; Pickled chinese cabbage, Mixed LAB

INTRODUCTION

Fermentation of perishable vegetables has thousand years of history; fermented vegetables are popular worldwide due to their special flavors attributed to a wide variety of fermentation technologies used(Palani et al., 2016; Zhao et al., 2018). Sauerkraut, namely, pickled Chinese cabbage (also called Chinese sauerkraut) and Sichuan pickles, are famous in the northeast and southwest areas of China, respectively(Liang et al., 2016). Other sauerkrauts, such as Guizhou sauerkraut and Korean kimchi (from Korea and Chinese Korean nationality), are processed with different methods. Pickled Chinese cabbage originated from three provinces in the northeast of China and became popular around the country; this traditional fermented vegetable is widely consumed in northeast of China and is prepared using Chinese cabbage(Zhao et al., 2018). Chinese cabbage is the main vegetable species utilized in autumn and winter in the northeast area of China because of its high nutrient content, low price, and long storage time(Cheng et al., 2016; Liang et al., 2016; Zhao et al., 2018). Chinese cabbage is processed into sauerkraut to extend the shelf life to more than four months(Choi et al., 2006). Homemade pickled Chinese cabbage, which has rich and mellow taste, is prepared by spontaneous fermentation in a big jar with salt, water, and Chinese cabbage placed under a big stone to ensure complete soaking for about one month(Yang et al., 2016). Given the increasingly popularity of this sauerkraut in China, its commercial production has begun and expanded(Zhao et al., 2018; Zhao et al., 2016). Manufacturers prefer spontaneous fermentation. However, few of them began to employ lactic acid bacteria (LAB) inoculation for fermentation of pickled Chinese cabbage, moreover this process shortens the fermentation time and degrades nitrites in pickled vegetables(Liang et al., 2016; Xia et al., 2017). Considering that nitrite in fermented vegetables could accumulate and cause serious harm to human health and the increasing awareness to food security, people have focused on using LAB inoculation for fermentation of pickled cabbage(Deb et al., 2007; Yan et al., 2015).

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LAB are Gram positive and could be widely used in food fermentation(Meng et al., 2016). Numerous LAB of pickled Chinese cabbage juice fermented by spontaneous fermentation were isolated, such as *Lactobacillus plantarum, Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus fermentum*, *Lactobacillus oligofermentans*, *Lactobacillus sakei*, *Lactobacillus coryniformis*, and *Weissella*(Chao et al., 2009; Jung et al., 2011; Xiong et al., 2012). *L. plantarum* is the most common isolation from pickled Chinese cabbage and plays a dominant role in fermentation. *Ln. mesenteroides* has a positive effect on the fermentation quality and flavor of Korean kimchi and is the main functional microorganism in fermentation juice(Jung et al., 2011; Jung et al., 2012). *Ln. mesenteroides* could be the main starter culture in fermentation of Chinese sauerkraut and Sichuan pickles, followed by *Enterococcus faecalis, Lactococcus lactis,* and *Lactobacillus Zeae* in the middle stage of fermentation(Jung et al., 2011). *L. casei* 11MZ-5-1 could be used as starter culture in fermentation of pickled Chinese cabbage; the product showed increased Vc content, decreased nitrite accumulation, and lower pH than pickled Chinese cabbage prepared by natural fermentation(Du et al., 2018). For banana by-products, CG1 strain were isolated from bananas among 11 LABs to improved the fermentation quality of defective banana silage(Yang et al., 2022). All these findings reveal that LAB fermentation of vegetable and fruit is a healthy option and produced high concentrations of volatile organic compounds(Rajendran et al., 2023).

Different LAB species were isolated from spontaneous fermentation of pickled Chinese cabbage and were found to contribute the rich flavor of the product(Jung et al., 2012). Scholars have reported that LAB isolates can improve fermentation indices and focused on inoculation by a single strain(Du et al., 2018; Zhao et al., 2018). In the present study, 13 LAB stains were isolated from pickled Chinese cabbage prepared by traditional spontaneous fermentation. Eleven isolations were screened for the following fermentation experiments according to their physiological biochemical characteristics. The indices of pickled Chinese cabbage inoculated by a single isolate were measured. Results indicated that the indices of X1, M2, and D2 were superior to the other strains. Therefore, the three stains were mixed for fermentation of pickled Chinese cabbage. The sensory quality and other indices of the product were then compared with those prepared by single inoculation and spontaneous fermentation.

MATERIALS AND METHODS

Isolation of LABs from pickled Chinese cabbage prepared by spontaneous fermentation

Chinese cabbage samples prepared by spontaneous fermentation were collected from seven different areas of Harbin, Heilongjiang Province, China. The leaf surface was sterilized, ground, and made into juice. The extract diluted from 10^0 to 10^{-8} by sterilization was placed on agar plates containing MRS medium. The plates were incubated at 37 °C for 48 h. The medium (1 L) comprised the following: 10 g of peptone, 8 g of beef extract, 4 g of yeast extract, 20 g of glucose, 2 g of $(NH_4)_2HC_6H_5O_7$, 5 g of $CH_3COONa \cdot 3H_2O$, 1 mL of Tween 80, 2 g of $K_2HPO_4 \cdot 3H_2O$, 0.58 g of $MgSO_4 \cdot 7H_2O$, and 0.25 g of $MnSO_4 \cdot H_2O$, with pH ranging from 6.2 to 6.4.

Identification of isolated strains

The isolated strains from the MRS medium plates were subjected to morphological characterization and Gram staining. The isolates were amplified with 16S rDNA and *rpoD* by PCR, with 25 μL reaction mixture containing 10.5 μL of dH_2O, 12.5 μL of PCR Mix (2X), 1 μL of isolation template, and 1 μL of each primer (forward, reverse). The PCR amplification procedure was set as follows, 94 °C for 5 min: 94 °C for 1 min, 58 °C for 30 s, 72 °C for 2 min for 30 cycles, and finally 72 °C for 10 min. The primers of 16S rDNA and *rpoD* are listed in Supplemental Table 1.

Biochemical characterizations of the isolates

All the isolates were cultivated on MRS medium, and the pH of the culture was detected every two hours until reaching 24 h. For acid production, the 24 h culture was titrated with phenolphthalein. Acid resistance was determined by the light absorption values of OD_{600} for all isolates cultivated in MRS for 24 h under different pH levels of 3.5, 4, 4.5, 5.5, and 6.5. Nitrate-reducing properties were determined by the survivability of the isolates on nitrate agar slants(Singh et al., 2013). The isolates were also evaluated in terms of physiological and biochemical indexes such as oxidase(Cobos et al., 2017), catalase activity(Singh et al., 2013), methyl red(Kim et al., 2017) and amylohydrolysis(Yarullina et al., 2015).

Preparation and inoculation into pickled Chinese cabbage

"Dongnong 909" Chinese cabbage samples were harvested in the experimental field of Northeast Agricultural University. The coat leaves were removed and lay up for 5–7 days. The vegetables were washed, dried, and placed into a big jar with 2% NaCl and 4% inoculum` by total weight of Chinese cabbage. Finally, the jar was filled with water to immerse the Chinese cabbage.

Characteristics of pickled Chinese cabbage

The pH of the fermentation juice of pickled Chinese cabbage was directly tested by pH meter (Sartorius PB-10). The pickled Chinese cabbage was ground for determining the contents of total acidity, soluble sugar, Vc(Zhao et al., 2018), nitrate and nitrite, and cellulose. The total acidity (lactic acid) content was determined by titration with phenolphthalein. Soluble sugar was tested by Anthrone colorimetry(Roe and Dailey, 1966). Nitrate content was measured by naphthalene diamine hydrochloride spectrophotometry as described in previous reports(Pu et al., 2001). The Anthrone colorimetry was also used to analyze cellulose content in Chinese sauerkraut(Herrmann et al., 1988).

RESULTS

Isolation and morphological identification of *LAB* from pickled Chinese cabbage

Seven samples of spontaneous fermentation pickled Chinese cabbage from different areas of Harbin, Heilongjiang province, China, were used for LAB isolation. A total of 13 LAB were screened by dissolving calcium ring method (Yan et al., 2015). As shown in Supplemental Fig. 1 and Supplental Table 2, the isolates were Gram positive and had smooth, white colonies and named as X1, X2, M1, M2, H2, H6, H7, W1, G3, Y2, Y3, Y4, and D2. Nine of these isolates were rhabditiform in shape, and the remaining (Y2, Y3, Y4, and D2) were globular.

Molecular identification of LAB isolates

X1, X2, M1, M2, H2, H6, H7, W1, G3, Y2, Y3, Y4, and D2 isolates were amplified by the primers of 16s rDNA, and the products were sequenced and inputted on the NCBI database. X1, X2, M1, M2, W1, G3, and H2 shared high homology to *L. plantarum*, as shown in Supplemental Table 3. H6 and H7 have 100% and 99% similarity to *Lactobacillus*, respectively. Y2, Y3, Y4, and D2 belonged to *Ln. mesenteroides*, which shared 99%, 100%, 100%, and 99% homology, respectively. For further confirmation of the results, another protein-coding gene, namely, *RNA polymerase sigma factor* (*rpoD*), was used. In our results, the *rpoD* gene of the 13 strains was amplified by PCR and sequenced. The results were inputted into the NCBI database. Nine of the strains (X1, X2, M1, M2, H2, H6, H7, W1, and G3) shared high similarity to *L. plantarum*, and the rest were homogeneous to *Ln. mesenteroides*.

Physiological and biochemical characteristics of the isolates

All of the 13 LAB isolates produced acid without gas, were positive for methyl red test and oxidase test and negative for catalase reaction, starch hydrolysis, and arginine dehydratase. The results are listed in Supplemental Table 4.

Biochemical characteristics of LAB isolates

Isolated strains X1, X2, M1, M2, W1, G3, and H2 had similar growth tendency in MRS medium, showing rapid continuous growth for 18 h and final OD_{600} values of above 1.4 after cultivation for 24 h. The growth curves of H6 and H7 were almost the same, reaching final $OD_{600} = 1.3$ after 24 h. Y2, Y3, Y4, and D2 slowly grew in the first 16 h and were identified as *L. mesenteroides*; their final concentrations were lower than those of the other strains (Fig. 1A). These results revealed that the most fertile strains were X1 and X2.

The 13 isolates were incubated in MRS (pH = 6) for 24 h, and pH was measured for calculating acid production rate. The acid production rates of X1, X2, M1, and W1 (2.79%, 2.70%, 2.79%, and 2.70%, respectively) were higher than those of the other strains. The acid production rates of Y2, Y3, Y4, and D2 were low (Fig. 1B). The 13 strains were cultivated in MRS for 24 h with different pH levels of 3.5, 4, 4.5, 5.5, and 6.5. The highest light absorption values of these strains were found at pH 6.5, which suggested that this pH was the most suitable for the strains (Fig. 1C). Each strain could hardly grow at pH 3.5, and their growth was inhibited at pH = 4–4.5 (Fig. 1C). Furthermore, X1, G3, and D2 demonstrated minimum changes in light absorption values between pH 4 and 6.5, which revealed their better resistance to low pH (Fig. 1C). Adding 2%–4% NaCl in MRS did not inhibit the growth of the strains (Fig. 1D). No strain survived in 8% NaCl MRS, and the growth of all the isolates was inhibited in 6% NaCl MRS (Fig. 1D). The results showed that the salt tolerance of the strains was arranged from strong to weak as G3, H7, H6, H2, M2, W1, Y3, Y4, X1, D2, M1, X2, and Y2(Fig. 1D).

Nitrite degradation characteristics of 13 LAB

Nitrite content is directly related to food security and thus should be evaluated. For nitrite degradation characteristics, each strain was cultivated in MRS with 10 mg/L $NaNO_2$ for 24h and the nitrite content was measured. Y4 and D2 exhibited the highest nitrite degradation rates, followed by Y2, Y3, H7, H6, X1, M2, X2, W1, G3, H2, and M1. Based on the biological characteristics, 8 strains (X1, M1, M2, WI, G3, H7, Y4, and D2) were selected for subsequent fermentation experiments of Chinese sauerkraut (Fig. 2).

Physicochemical indices of pickled Chinese cabbage fermented with single isolate inoculated

In the present work, Chinese cabbage was fermented with single inoculation of X1, X2, M1, M2, W1, G3, H2, H6, H7, Y2, Y3, Y4, and D2 to explore their fermentation performance. After 30 days of fermentation, physiological indices were measured. The pH of the fermentation liquor was 6 before inoculation, and the juice pH of M2, W1, and H7 inocula reached 3.5 after fermentation. M1 and Y4 obtained the highest pH of 3.8 (Supplemental Fig. 3). The pickled Chinese cabbage inoculated by M2 had the highest acid content (lactic acid), followed by H7, D4, and G3 and M1 were the lowest (Fig. 3A).

Pickled Chinese cabbage inoculated with Y2 had the highest content of soluble sugar, which indicated that

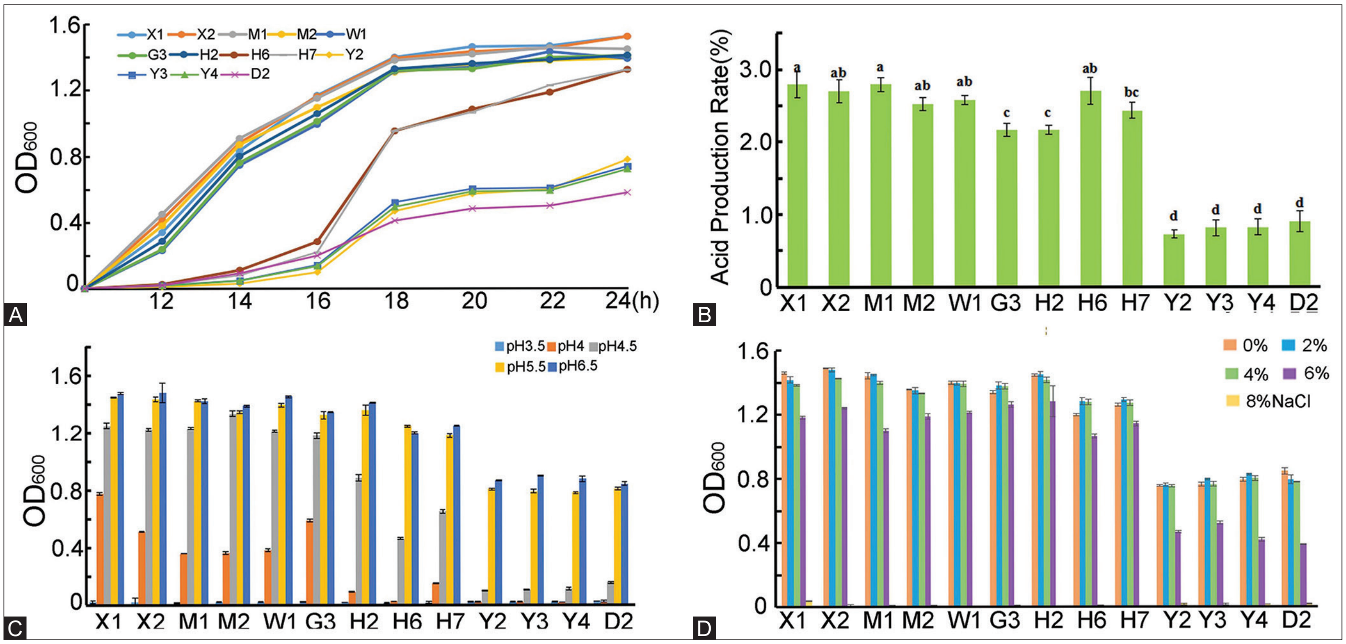

Fig 1. Biological characteristics of 13 LAB isolates (X1, X2, M1, M2, W1, G3, H2, H6, H7, Y2, Y3, Y4, and D2). A, Growth curves in MRS medium; B, Acid-producing rates; C, Growth status in MRS medium with pH 3.5, 4, 4.5, 5.5, and 6.5; D, Growth status in MRS medium with 0%, 2%, 4%, 6%, and 8% NaCl.

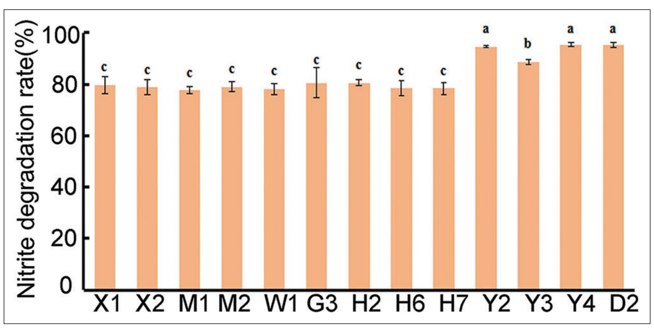

Fig 2. Nitrite degradation rates of 13 LAB.

Y2 consumed less soluble sugar than the other strains. By contrast, M2 had the lowest soluble sugar content. The soluble sugar contents in the other strains showed no significant difference (Fig. 3B). The present results showed that the Vc contents in pickled Chinese cabbage fermented by M2 were the highest (3.09 mg/100 g), and X1 and D2 also had high Vc contents reaching 3.04 and 2.99 mg/100g, respectively(Fig. 3C). The fiber contents in Chinese cabbage were crucial to the taste and flavor of pickled Chinese cabbage and were affected by fermentation. The highest fiber content was found in pickled Chinese cabbage inoculated with W1, followed by D2. The fiber content in Chinese sauerkraut fermented by G3 was the lowest(Fig. 3D).

In the present study, the nitrite contents in pickled Chinese cabbage were measured and compared. The highest content was found in the product inoculated with M1, reaching 1.33 mg/kg, and the lowest was detected in the product with H7, reaches 1.20 mg/kg. The nitrite contents in the products with X1, M2, Y4, and D2 were slightly higher than that in the product with H7, but no statistical difference was observed (Fig. 3E).

Characteristics of pickled Chinese cabbage inoculated with mixed LAB

Three superior strains (X1, M2, and D2) were selected and mixed at 1:1:1 to improve the fermentation characteristics of pickled Chinese cabbage. After 30 days of fermentation, the pickled Chinese cabbage prepared by spontaneous fermentation (CK) and inoculation with mixed strains (mixed) were compared. The acid contents in Chinese sauerkraut inoculated with the mixed strains (0.85%) were significantly higher than those in CK (0.713%)(Fig. 4A). The soluble sugar content in mixed (0.16%) was lower than that in CK (0.22%) (Fig. 4B). These results were consistent with previous data of acid content. The Vc and fiber contents in the mixed (4.1 mg/100 mg and 0.26%, respectively) were significantly higher than those in CK (2.9 mg/100 mg and 0.22%, respectively)(Fig. 4C), thereby affecting the quality of pickled Chinese cabbage. The nitrite content in Chinese sauerkraut prepared by mixed strains decreased to 0.69 mg/kg after fermentation for 30 days(Fig. 4D), which was lower than that in CK (2.07 mg/kg, Fig. 4E) and sauerkraut prepared by single-strain fermentation. All these results showed the superior characteristics of pickled Chinese cabbage inoculated by the mixture of the three strains (X1, M2 and D2) over the other samples.

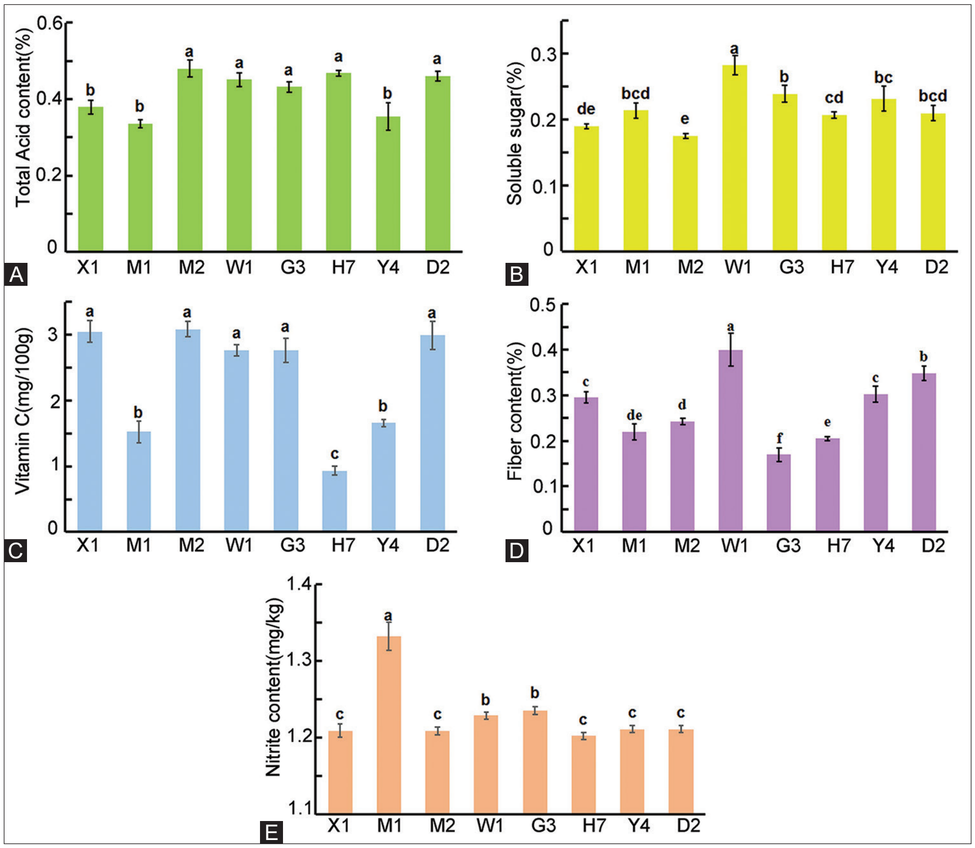

Fig 3. Characteristics of pickled Chinese cabbage inoculated with single isolates (X1, X2, M1, M2, W1, G3, H2, H6, H7, Y2, Y3, Y4, and D2). A, Acid contents. B, Soluble sugar content. C, Vc contents. D, Cellulose contents. E, Nitrite contents.

Sensory evaluation of pickled Chinese cabbage

Sensory evaluation of fermented pickled Chinese is crucial to characterize the commodity. Although the characteristics of pickled Chinese cabbage inoculated by the mixed strain were excellent, the sensory quality remained to be measured (Fig. 5). Aroma, crispness, sourness, and color were evaluated by scoring (total score of 12; good = 3, medium = 2, bad = 1). The sensory quality was assessed among pickled Chinese cabbage prepared by inoculation with single strain, mixed strain and spontaneous fermentation (CK). The Chinese sauerkraut with the mixed strain had the highest score, and the scores of the samples with single strain were higher than those of CK, except for G3.

DISCUSSION

Pickled Chinese cabbage is polular traditional fermented vegetables in China, which derive from northeast parts (Palani et al., 2016; Zhao et al., 2018). Chinese sauerkraut is favored by consumers for its strong and unique flavor, which were caused by the abundant secondary metabolites of the LAB during spontaneous fermentation process(Liang et al., 2016; Zhao et al., 2018). *Lactobacillus plantarum* and *L. mesenteroides* were reportes as the starters culture for pickled Chinese cabbage(Yang et al., 2016). In the present work, Chinese cabbage was fermented with single inoculation of X1, X2, M1, M2, W1, G3, H2, H6, H7, Y2, Y3, Y4, and D2 to explore their fermentation performance. After 30 days of fermentation, physiological indices were measured. The pH of the fermentation liquor was 6 before inoculation, and the juice pH of M2, W1, and H7 inocula reached 3.5 after fermentation. M1 and Y4 obtained the highest pH of 3.8 (Supplemental Fig. 3). The pickled Chinese cabbage inoculated by M2 had the highest acid content (lactic acid), followed by H7, D4, and G3 and M1 were the lowest (Fig. 3A). *Ln. mesenteroides* was the main functional microorganism to produce fermentation quality and flavor for kimchi (Jung et al., 2011; Jung et al., 2012).

LAB isolated from spontaneous pickled Chinese cabbage

In this study we isolated 13 LAB from traditional spontaneous fermentation pickled Chinese cabbage from 7 different areas in Harbin Heilongjiang province China. Morphology, physiological and biochemical identification of these 13 isolates were performed. They were smooth and white producing acid without gas, positive for Gram, methyl red test and oxidase test and negative for catalase reaction, starch hydrolysis and arginine dehydratase, named as X1, X2, M1, M2, H2, H6, H7, W1, G3, Y2, Y3,

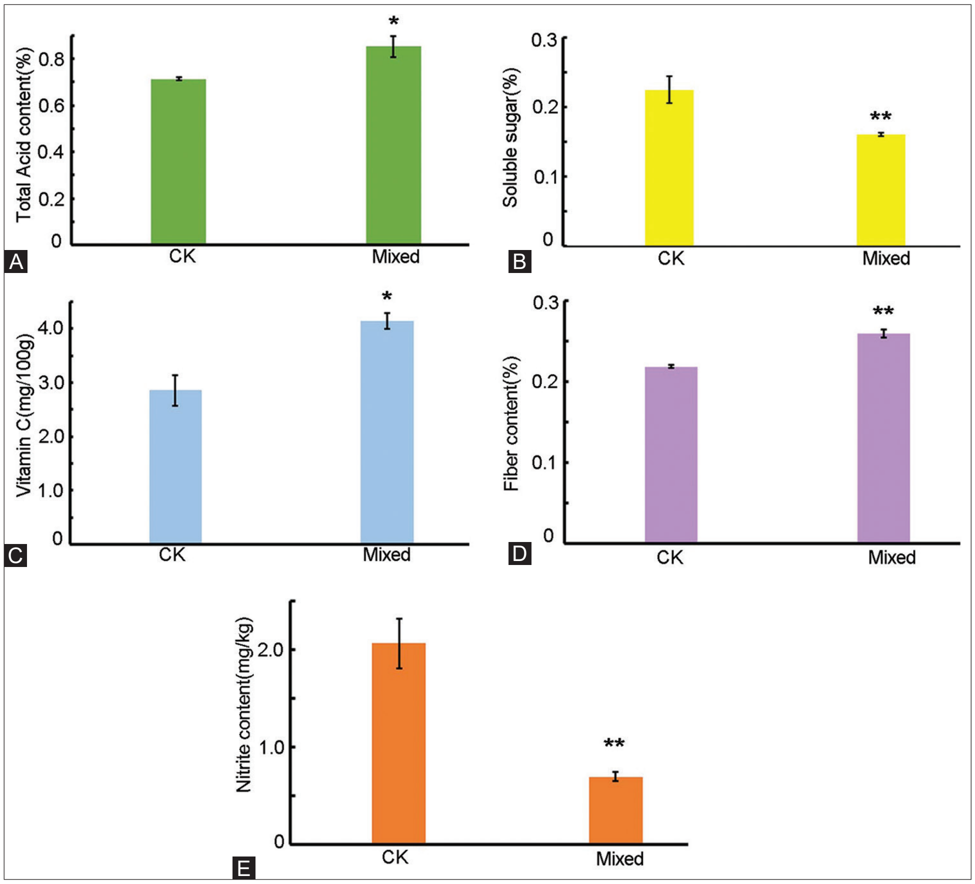

Fig 4. Characteristics of pickled Chinese cabbage inoculated with the mixture of X1, M2, and D2. A, Total acid content. B, Soluble sugar content. C, Vc content. D, Cellulosecontents. E, Nitrite contents. F, Nitrate contents. '*' means p < 0.05, and '**' means p < 0.01.

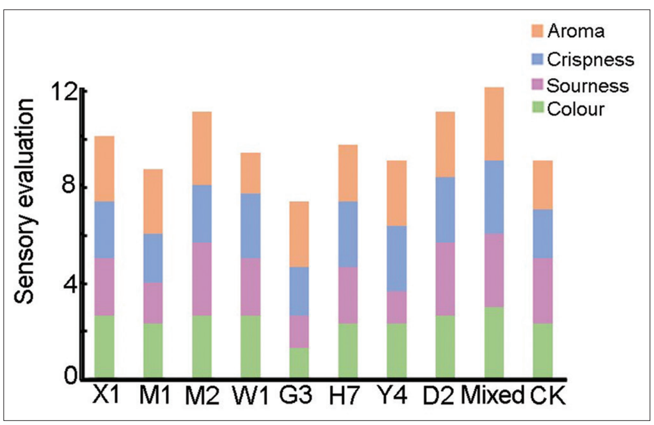

Fig 5. Sensory evaluation of pickled Chinese cabbage. The Chinese sauerkraut prepared by inoculation with X1, M1, M2, W1, G3, H7, Y4, and D2, inoculation with the mixed strain, and spontaneous fermentation were assessed.

Y4, and D2. Meanwhile molecular identifications were done, including 16s-rDNA and *rpoD*. The *rpoD* gene shares conservative sequences in various eubacteria such as *E. coli*, *Bacillus subtilis* and *Azospirillum* spp., which are commonly used for molecular identification (Araya et al., 1995; Maroniche et al., 2017; Tanaka et al., 1988). Most of the isolations (9/13) shared more than 98% similarity to *L. plantarum*, and Y2, Y3, Y4, and D2 were homogeneous to *Ln. Mesenteroides* by 16s rDNA. The identifications of Y2, Y3, Y4, and D2 by *rpoD* gene also showed the highest homologous to *Ln. Mesenteroides,* and the rest nine stains belonged to *L. plantarum*. For further exploration the growth characteristics of the 13 strains, the growth curve, acid production, the acid resistance and salt resistance of them were detected. *L. plantarum* X1, X2, M1, M2, W1, G3, and H2 grew rapidly after inoculation, and H6 and H7 were slower in the first 16 hours and then rapidly rise. *Ln. Mesenteroides* Y2, Y3, Y4, and D2 grew slower than *L. plantarum* and finally reached a lower cell concentration than above *L. plantarum*in. Among these LAB, *Lactobacillus plantarum* played a dominant role in the fermentation of Chinese cabbage because of its acid production and resistance.The acid production rates of *Lactobacillus plantarum* were higher than *Ln. Mesenteroides* (Fig. 1B). Given that LAB will produce a considerable amount of acid during fermentation, screening acid-resistant strains is necessary to ensure the quality of pickled Chinese cabbage. Our results showed X1, G3, and D2 had better resistance to low pH (Fig. 1C), and the salt tolerance of the strains was arranged from strong to weak as G3, H7, H6, H2, M2, W1, Y3, Y4, X1, D2, M1, X2, and Y2(Fig. 1D).

Improvements of pickled Chinese cabbage fermentation index by single LAB and their mixture

LAB are widely used in fermented foods and could produce acids during fermentation (Meng et al., 2016). For exploring the utilization of the isolated LAB in vegetable fermentation, each of them was inoculated as the single inoculum for the fermentation of pickled Chinese cabbage. The total acid content, soluble sugar, Vc content and fibrin content were detected. The acid content (lactic acid) of the pickled Chinese cabbage inoculated by M2 were highest among other single strain(Fig. 3A). LAB use the carbohydrate of Chinese cabbage as carbon source for rapid multiplication and simultaneous production of lactic acid and other flavor substances(Ishii et al., 2017; Jeske et al., 2018). M2 pickled Chinese cabbage had the lowest soluble sugar content, indicating that it consumed the most sugar and produced a large amount of flavor substances. Fresh vegetables have high Vc, which is beneficial to body health. However, Vc decreased with increasing time for fermentation of Chinese cabbage, as reported in previous studies(Du et al., 2018; D. Zhao et al., 2018). Our results showed that the Vc contents in pickled Chinese cabbage fermented by M2 were the highest. Dietary fiber, which has become increasingly popular in recent years, has considerable benefits to the human body(Zhang et al., 2018). Chinese cabbage has high amounts of dietary fiber, including soluble and insoluble dietary fiber(Wang and Ma, 2017). Microbiology plays a positive role in fiber degradation(Cheng et al., 2018). The fiber content of W1 pickled Chinese cabbage was the highest, followed by D2. And then three superior strains (X1, M2, and D2) were mixed together as the inocula with the concentration of 1:1:1 for the fermentation of pickled Chinese cabbage. The acid, Vc, fiber contents and sensory evaluation of mixed strains Chinese sauerkraut were significantly highest among CK and even all that samples fermented by single strain. These results indicated the dominant acidity of pickled Chinese cabbage prepared by mixed inoculation. However, the soluble sugar content of mixed Chinese sauerkraut was lowest among CK and sauerkraut prepared by single-strain fermentation.

Comparation of nitrite contents by different fermentationsed inoculum

Nitrate and nitrite contents of food are a serious problem that threatens food safety, especially for fermented food vegetables. During fermentation, nitrate is converted into nitrite, which is implicated to increased risk of gastrointestinal cancer in humans(Hord et al., 2009; Kumar et al., 2017). Each isolated strain was detected for nitrite degradation characteristics, which showed that Y4 and D2 exhibited the highest nitrite degradation rates. The nitrite content of Chinese sauerkraut prepared by mixed strains was lowest than both CK and these prepared by single-strain fermentation. All these results showed the superior characteristics of pickled Chinese cabbage inoculated by the mixture of the three strains (X1, M2 and D2) over the other samples.

CONCLUSIONS

In this work, 13 LAB, including *L. plantarum* and *Ln. mesenteroides*, were isolated from pickled Chinese cabbage prepared by traditional spontaneous fermentation. Chinese cabbage was inoculated with single strain and their mixture (X1, M2, and D2) and fermented for 30 days. The sample inoculated with the mixture had the highest acid and Vc contents, the lowest nitrite content, and medium fiber content. Furthermore, the sensory quality of pickled Chinese cabbage fermented with the mixed inocula was the best compared with products prepared with single strain and spontaneous fermentation.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Authors contribution

Y.L. and M.Y. contributed to methodology, investigation, for fifield experiments, Y.L. analyzed the data and results, wrote the manuscript; D.L.L. for the experiments of supplementary data and the analysis of supplementary data; Y.W.Z. designed the experiments and supervise the manuscript.

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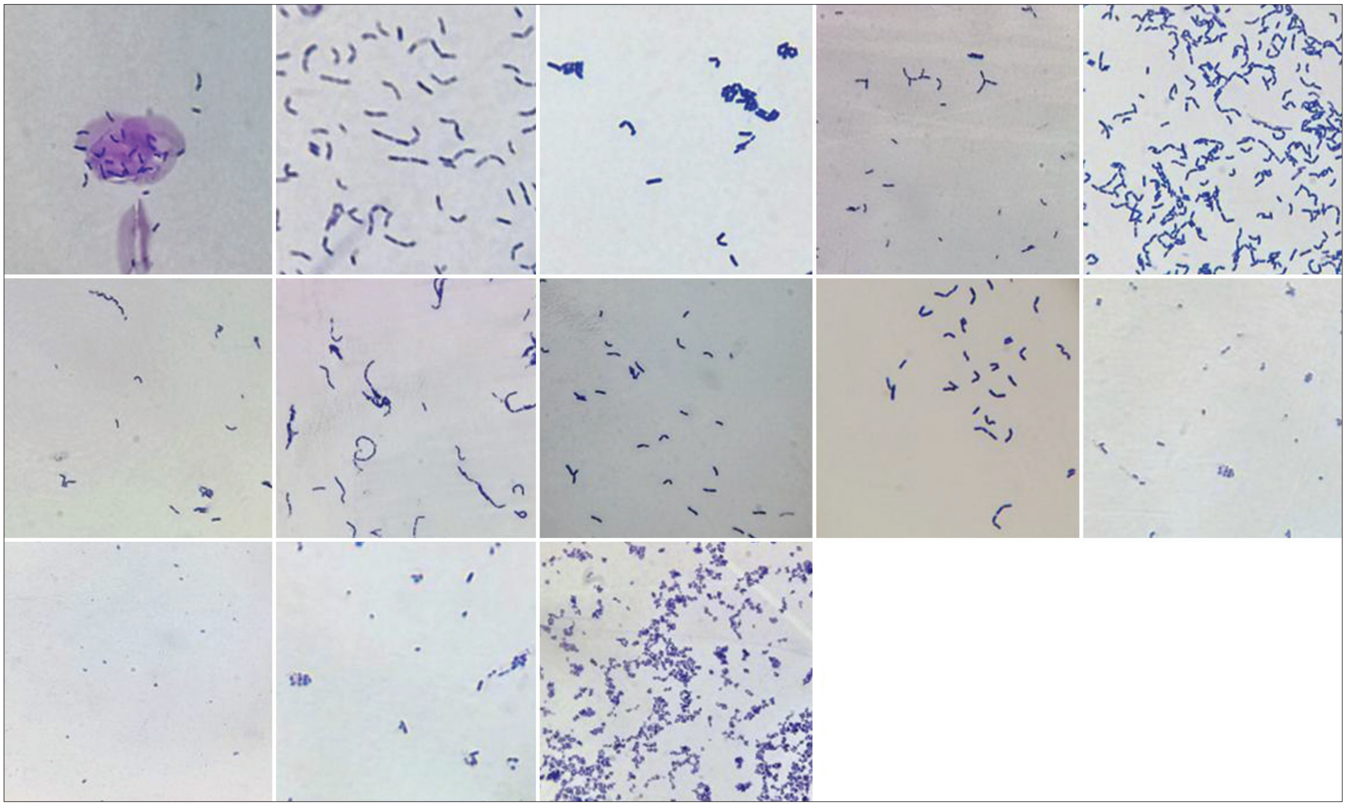
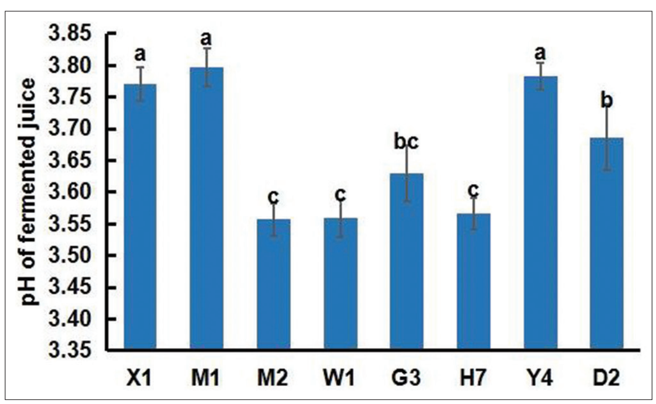
SUPPLEMENTARY FIGURES



Supplemental Fig 1. Gram stain of the 13 isolated strains



Supplemental Fig 2. The fermentation juice pH of the13 isolated strains

SUPPLEMENTARY TABLES

Supplental Table 1: The primers of molecular identification

Name	Forward Sequence	Reverse Sequence
16s	5'-AGAGTTTGATCCTGG CTCA G-3'	5'-GGTTACCTTGTTACGACT T-3'
LPrpoD	5'-GATGCTGGGATTAG TGTTG-3'	5'-GAGGTTGCGTCTTGGTC T-3'
LMrpoD	5'-GAGTACGACAAGGC AACC3'	5'-CAAATCCATTTCAGCAC C-3'

LPrpoD means the *lactobacillus plantarum rpoD* gene ; *LMrpoD* means the *Leuconostoc mesenteroides rpoD* gene

Supplental Table 2: The morphological observation of the bacterial strains

Strains	Shape	Colour	Transparency	Surface morphology	Individual	Gram staining
X1	circular	white	opaque	smooth; protruding	rhabditiform	G+
X2	circular	white	opaque	smooth; protruding	rhabditiform	G+
M1	circular	white	opaque	smooth; protruding	rhabditiform	G+
M2	circular	white	opaque	smooth; protruding	rhabditiform	G+
H2	circular	white	opaque	smooth; protruding	rhabditiform	G+
H6	circular	white	opaque	smooth; protruding	rhabditiform	G+
H7	circular	white	opaque	smooth; protruding	rhabditiform	G+
W1	circular	white	opaque	smooth; protruding	rhabditiform	G+
G3	circular	white	opaque	smooth; protruding	rhabditiform	G+
Y2	circular	white	opaque	smooth; flat	globular	G+
Y3	circular	white	opaque	smooth; flat	globular	G+
Y4	circular	white	opaque	smooth; flat	globular	G+
D2	circular	white	opaque	smooth; flat	globular	G+

Supplental Table 3: The BLAST results of the strains on NCBI by 16s rDNA

Strains	Identification	Similarity
X1	*Lactobacillus plantarum*	100%
X2	*Lactobacillus plantarum*	100%
M1	*Lactobacillus plantarum*	100%
M2	*Lactobacillus plantarum*	100%
H2	*Lactobacillus plantarum*	100%
H6	*Lactobacillus*	99%
H7	*Lactobacillus*	100%
W1	*Lactobacillus plantarum*	100%
G3	*Lactobacillus plantarum*	98%
Y2	*Leuconostoc mesenteroides*	99%
Y3	*Leuconostoc mesenteroides*	100%
Y4	*Leuconostoc mesenteroides*	100%
D2	Leuconostoc mesenteroides	99%

Supplental Table 4: Physiological and biochemical characteristics of the bacterial strains

Strains	Catalase reaction	Starch hydrolysis	Methyl red test	Arginine production of ammonia	Acid producing	Gas producing	Oxidase test
X1	–	–	+	–	+	–	+
X2	–	–	+	–	+	–	+
M1	–	–	+	–	+	–	+
M2	–	–	+	–	+	–	+
H2	–	–	+	–	+	–	+
H6	–	–	+	–	+	–	+
H7	–	–	+	–	+	–	+
W1	–	–	+	–	+	–	+
G3	–	–	+	–	+	–	+
Y2	–	–	+	–	+	–	+
Y3	–	–	+	–	+	–	+
Y4	–	–	+	–	+	–	+
D2	–	–	+	–	+	–	+