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Effects of cultivation conditions on the diversity of microbes involved in the conversion of rice straw to fodder

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Abstract

To confirm the optimum cultivation conditions for analyzing lactic acid bacterial communities and to provide the cultivation foundation for lactic acid bacterial communities that were used to convert straw into fodder, fermented rice straw was inoculated into 13 different broths. After 48 h of cultivation, pH values, volatile products, and microbial diversity were analyzed. Except for LAB broth, the pH values of the other broths could decrease to approximately 4.5. GC/MS analysis showed that lactic acid in Tomato MRS broth, MRS broth, LAB broth, and Tomato juice broth was higher than that in the other broths. DNA concentration analysis showed that the counts of microbes in Tomato MRS broth were 2.5 times of those in other broths and that tomato juice favored the reproduction of the microbes. Denaturing gradient gel electrophoresis (DGGE) analysis showed that the number of lactic acid bacterial species in HYA broth, Tomato juice broth, and Tomato MRS broth were higher than those in the other broths.

Key words: straw; cultivation condition; microbial diversity; DGGE

Introduction

In China, the annual yield of straw and stalk of the main crops is about 604×10^6 t, which includes 115.4×10^6 and 139.6×10^6 t of wheat and rice straw, respectively. About 50% of this biomass resource is burned for cooking and heating, or left, or combusted directly on the lands, which lead to resources wasting and also cause environmental pollution (Liu *et al.*, 2005). Although tremendous efforts have been made to transform these wastes into value-added products, this kind of resources has not yet been fully utilized (Pan and Sano, 2005).

In general, there is a decrease in the number of feeding ruminants in North China during the winter season. The transformation of straw to fodder considerably reduces the accumulation of waste in the environment, thereby serving as a means to transform wastes into value-added products. However, straw is mainly composed of cellulose, hemicellulose, and lignin, which are hard to be digested and assimilated by the ruminants in feedlots (Kim *et al.*, 2005). In particular, the successful fermentation of wheat straw and rice straw by the epiphytic microbes is difficult (Gao *et al.*, 2005). A previous study showed that the silage quality of grass, such as alfalfa, could be improved by inoculating lactic acid bacterial community (Wang *et al.*, 2004). The fermentation quality of straw could be improved by this means.

On account of the complex composition of microbial communities, their analysis using the traditional culture methods, such as enrichment and isolation, is quite difficult. Compared with the traditional culture methods, the molecular approaches are convenient and efficient for microbial community analyses. Denaturing gradient gel electrophoresis (DGGE), one of the molecular methods, exhibits its advantage in analyzing artificial microbial communities with high cellulose-degradation ability (Haruta *et al.*, 2002). It can directly provide significant information of advantageous species in the DGGE profiles without the need for cultivation. It is reproducible and easily operable. Furthermore, the data from the DGGE profiles can be used as a criterion for controlling a microbial community (Kato *et al.*, 2004). However, Muyzer *et al.* (1997) had reported that only when the percentage of a particular microbe occupied more than 1% of the microbial community, they could be effectively detected using DGGE. According to the results of Wang *et al.* (2006), when DGGE was used to analyze the lactic acid bacterial community obtained by enrichment cultures, the profiles showed two advantageous species of the total seven species of bacteria. It showed that traditional enrichment method could not be ruled out in the microbial community analysis, indicating that the cultivation condition may have an effect on the analytical results of DGGE. As a result, for a microbial community, if cultivation conditions are suitable, the microbes in the community showed a uniform increase, and the maximum advantage of DGGE can be achieved.

In the present study, 13 different broths were chosen as the cultivation medium and inoculated with fermented rice

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straw, and DGGE was performed to analyze the microbial composition of different cultures. The main objectives of this study were to select more suitable broths for the uniform growth of lactic acid bacteria and provide the cultivation foundation for lactic acid bacterial community that were used to convert straw into fodder.

1 Materials and methods

1.1 Ensiling

Rice straw was obtained from the experimental farm of China Agricultural University, Beijing, China. After harvest, the rice straw was air-dried. It was chopped to about 1 cm size for fermentation. Glucose (Beijing Chemical Reagent Company, China) was used to adjust the water-soluble carbohydrate level of the rice straw to 10.0% dry matter (dm). Deionized water was sprayed on the rice straw, and the final moisture was approximately 70% of the mixtures. The mixtures were ensiled in 100-ml test-tube silos and were allowed to ferment under anaerobic condition at 30°C (Yang *et al.*, 2006). Three silos were taken on the day 4 of fermentation for the following analysis. The chemical compositions of the rice straw before and after fermentation are listed in Table 1.

1.2 Media and cultivation conditions

1.2.1 Media

The broths were prepared as follows:

LAB broth (LAB): beef extract 10 g, yeast extract 10 g, lactose 20 g, Tween80 1 ml, $CaCO_3$ 10 g, KH_2PO_4 2 g, distilled water 950 ml, pH 6.6 (Xu *et al.*, 1994).

MRS broth (MRS): peptone 10 g, beef extract 10 g, yeast extract 5 g, glucose 20 g, KH_2PO_4 2 g, triammonium citrate 2 g, sodium acetate 5 g, Tween80 1 ml, $MgSO_4 \cdot 7H_2O$ 0.58 g, $MgSO_4 \cdot 4H_2O$ 0.25 g, distilled water 1000 ml, pH 6.2–6.4 (Horn *et al.*, 2005).

Tomato MRS broth (T-MRS): 200 ml of tomato juice was added to MRS broth and the final volume was made up to 1000 ml (Tan *et al.*, 2001).

GYP broth (GYP): glucose 30 g, yeast extract 5 g, tryptone 3 g, distilled water 1000 ml, pH 6.8 (Kozaki *et al.*, 1992).

Modified Chalmers broth (M-Ch): peptone 3 g, beef extract 3 g, yeast extract 3 g, glucose 20 g, distilled water 1000 ml, pH 6.0 (Vanos and Cox, 1986).

Broth from Shanghai foodstuff supervisor (Shh): peptone 3 g, beef extract 3 g, yeast extract 3 g, lactose 20 g, distilled water 1000 ml, pH 6.0 (Xu *et al.*, 1994).

Rogosa broth (Rogosa): tryptone 10 g, yeast extract 5 g, glucose 10 g, arabinose 5 g, sucrose 5 g, KH_2PO_4 6 g, ammonium citrate 2 g, sodium acetate 15 g, Tween80 1 ml, $MgSO_4$ 0.57 g, $MgSO_4$ 0.12 g, $FeSO_4$ 0.03 g, distilled water 1000 ml, pH 5.3–5.5 (Rogosa *et al.*, 1951).

Broth for lactic acid bacteria (Zhou): glucose 50 g, peptone 5.0 g, yeast extract 2 g, KH_2PO_4 2 g, $CaCO_3$ 3 g, pH 7.0, distilled water 1000 ml (Zhou *et al*., 2002).

Nutritive milk casein broth (N-milk): peptonized milk 7 g, glucose 1 g, milk casein 3 g, distilled water 1000 ml, pH 6.6 (Li *et al.*, 1991).

HYA broth (HYA): beef extract 1 g, glucose 2.5 g, galactose 2.5 g, lactose 5.0 g, tryptone 10 g, distilled water 1000 ml, pH 7.0 (Li *et al*., 1991).

Tomato juice broth (T-juice): tomato juice 400 ml, peptone 10 g, peptonized milk 10 g, distilled water 1000 ml, pH 6.0–6.2 (Chen, 1995).

Standard methods broth (SM): beef extract 2.5 g, tryptone 5 g, glucose 1 g, distilled water 1000 ml, pH 7.0 (Bradshaw, 1979).

Whey broth (Whey): fat-free milk (1000 ml) was heated to 90°C and then diluted hydrochloric acid (10%, v/v) was added to heated fat-free milk. When white sediments and supernatant fluid were formed, the addition of hydrochloric acid was stopped. The volume of hydrochloric acid added was approximately 50–60 ml. The supernatant fluid was separated from the white sediments with absorbent cotton, and 10 g of peptone was added into the supernatant fluid. This fluid with peptone was the Whey broth with pH 6.0 (Li *et al*., 1991).

All the broths were autoclaved for 15 min at 121°C.

1.2.2 Culture methods

About 50 ml of broth was transferred into a 50-ml flask. Each broth had three triplicates. The fresh fermented rice straw of three silos was then mixed in a clean bench, and 2 g of mixtures were added into the flask and were cultured at 30°C for 2 d.

1.3 Analytical methods

1.3.1 Chemical analyses

The pH values were determined using HORIBA Compact pH meter (Model B-212, Japan). A total of 1.5 ml of cultures were transferred into 10-ml tube containing 3 ml of sterile water, and the mixtures were immerged for 30 min. After centrifugation and filtration (0.22 μm), the fluids were analyzed by gas chromatography mass spectrometry (GC/MS) method (Model GCMS-QP2010, Shimadzu, Japan): One microliter of fermented juice was injected into the capillary column (CP-Chirasil-Dex CB, 25 m×0.25 mm); split ratio was 20:1; injector temperature was 190°C; ion source temperature was 200°C; interface temperature was 200°C; detector voltage was 0.7 kV; and

Table 1 Chemical compositions of the rice straw before and after fermentation

Item	Moisture (%)	pH	WSC (% dm)	Ethanol	Glycol	AA	LA	Glycerol
				(% fm)				
Rice straw	7.0	6.4	9.4	/	/	/	/	/
Fermented straw	71.3	4.2	3.2	0.74	1.19	0.92	2.98	2.09

AA: acetic acid; LA: lactic acid; dm: dry matter; fm: fresh matter; WSC: water-soluble carbohydrate.

carrier gas was helium. The column head pressure was 75 kPa, and the column flow rate was 35.7 ml/min. Temperature program: initial temperature 50°C (for 1 min), and then increased at the rate of 10°C/min to 190°C, which was the final temperature (for 4 min). The data were analyzed by the Shimadzu GC/MS solution V2.4 data processing system. Each analysis was performed in triplicates.

Data were subjected to ANOVA by the general linear models procedure of Statistic Analysis System (Ver.6.12; SAS Inst., Cary, NC, USA).

1.3.2 Microbial communities analyses

For analyzing the complexity of the microbial communities from all the broths, the DNA of each broth treatment was extracted by the benzyl chloride method (Zhu *et al.*, 1993). The DNA concentration was determined using a DNA/RNA/protein analyzer (Biospec-mini, Shimadzu, Japan). The extracted DNA was diluted to the same concentration and was used as the template in PCR amplification. The primers used to amplify the V3 region of 16S rDNA were the following: 357F-GC, 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGG-CACGGGGGGCCTACGGGAGGCAGCAG-3′(*Escherichia coli* positions, 341 to 357), and 517R, 5′-ATTACCG-CGGCTGCTGG-3′ (*E. coli* positions, 517 to 534). The underlined sequence represents a GC clamp (Haruta *et al*., 2004). The initial DNA denaturation was performed at 95°C for 10 min, followed by 30 cycles of denaturation at 93°C for 1 min, annealing at 48°C for 1 min, and elongation at 72°C for 1 min 10 s and then a final elongation step at 72°C for 5 min.

The DGGE analysis was performed with the Dcode™ system (Bio-Rad Laboratories, Hercules, CA) according to Muyzer *et al.* (1993) and Wang *et al*. (2006). A total of 13 μl of PCR products was applied to 1-mm thick, 6%–12% (w/v) polyacrylamide gradient gels in a 0.5 × TAE electrophoresis buffer (20 mmol/L Tris-HCl pH 8.3, 10 mmol/L acetic acid, and 0.5 mmol/L EDTA), with 20%–60% denaturant gradient (where 100% is defined as 7 mol/L urea with 40% formamide). Electrophoresis was performed at a constant voltage of 200 V and a temperature of 61°C for 5 h. The V3 region bands of 16S rDNA on the gel were stained with SYBR® Green I (Pedro *et al.*, 2001). The bands on DGGE gels were observed under UV 302 nm light using the AlphaImager 2200 Imaging System (Alpha Innotech, USA). The DNA was recovered and reamplified with the primers 357F (5′-CCTACGGGAGGCAGCAG-3′) and 517R (5′-ATTACCGCGGCTGCTGG-3′) (Haruta *et al.*, 2004). Initial DNA denaturation was performed at 95°C for 10 min, followed by 25 cycles of denaturation at 93°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 1.5 min and then a final elongation step at 72°C for 5 min. The amplified fragments were purified using the high pure PCR product purification kit (Tiangen Biotech Co., LTD, China) according to the manufacturer's instructions. The purified PCR fragments were sequenced using the ABI 3730XL DNA Sequencer (Perkin Elmer) at SunBiotech Devoloping Center (http://www.sunbiotech.com.cn).

The sequence similarity searches were performed in the GenBank data library using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). The V3 region sequences of the 16S rDNA were compared with the sequences from strains deposited in GenBank. The sequence information was then imported into the CLUSTAL X software program for assembly and alignment (Thompson *et al.*, 1997). The phylogenetic tree was constructed by neighbor joining method (Saitou and Nei, 1987). The V3 region sequences of the 16S rDNA generated in this study were deposited in GenBank under accession numbers DQ334784 to DQ334801.

2 Results and discussion

2.1 Changes in pH values of different broths during cultivation

A total of 2 g of fermented rice straw was inoculated into 13 different broths. The pH values were determined at intervals 0, 12, 24, and 48 h of cultivation (Table 2). Changes in the pH values of the cultures mostly occurred during the 12 h of cultivation. However, the pH values of LAB, MRS, T-MRS, and Rogoza broths reduced more slowly than other broths during this period. The pH values of all the broths decreased with time from 12 h to 48 h.

Table 2 Changes in the pH values of different broths

Broth	pH_0	pH_{12}	pH_{24}	pH_{48}	$pH_0 - pH_{12}$	$pH_{12} - pH_{24}$	$pH_{24} - pH_{48}$
LAB	6.6	5.9±0.1[#]	5.2±0.1	4.7±0.2	0.7^{i*}	0.6^{d}	0.5^{ab}
MRS	6.2	5.4±0.0	4.5±0.1	4.1±0.0	0.8^{hi}	0.9^{c}	0.4^{ab}
T-MRS	6.2	5.3±0.1	4.1±0.1	4.0±0.1	0.9^{h}	1.2^{a}	0.2^{de}
GYP	6.8	4.2±0.0	3.7±0.1	3.5±0.1	2.6^{b}	0.5^{ef}	0.3^{cd}
M-Ch	7.2	4.8±0.1	3.8±0.0	3.6±0.0	2.4^{cd}	1.0^{b}	0.2^{d}
Shh	6.0	4.3±0.1	4.4±0.1	3.7±0.1	1.7^{f}	0.0^{h}	0.6^{a}
Rogosa	5.5	5.5±0.1	4.6±0.1	4.5±0.1	0.0^{j}	0.8^{c}	0.2^{de}
Zhou	7.0	4.5±0.1	3.7±0.1	3.4±0.1	2.5^{c}	0.8^{c}	0.4^{bc}
N-milk	6.6	4.3±0.0	4.0±0.1	3.9±0.0	2.3^{d}	0.2^{g}	0.2^{de}
HYA	6.8	4.4±0.1	3.8±0.0	3.8±0.1	2.4^{cd}	0.6^{de}	0.0^{e}
T-juice	6.2	4.4±0.1	4.0±0.0	3.8±0.1	1.8^{e}	0.4^{f}	0.3^{d}
SM	7.0	4.3±0.1	4.4±0.1	4.1±0.1	2.7^{a}	0.1^{h}	0.0^{e}
Whey	6.0	4.4±0.1	4.0±0.1	3.8±0.0	1.6^{g}	0.5^{ef}	0.2^{de}

[#]pH values indicate average ±*SD* (n=3) at time intervals of 0, 12, 24, and 48 h of cultivation; [*] ^{a,b,c,d} means followed by different superscripts are significantly different (P<0.05).

After 48 h of cultivation, the pH values decreased to less than 4.5 except LAB and Rogosa broths.

Decrease in the pH values of different broths indicates an increase in the synthesis of acids by the microbes. However, the pH values of LAB, MRS, T-MRS, and Rogoza broths decreased more slowly than other broths during the first 12 h of cultivation. This is because of the buffering capacity of K_2HPO_4 (MRS and T-MRS) or K_2HPO_4 (LAB and Rogosa), and this buffering capacity is maintained until 48 h of cultivation, which resulted in the differences in the composition of microbial communities in different broths (Section 2.3.2).

2.2 Analyses of volatile products

2.2.1 Effects of broth compositions on volatile products

As the volatile products were produced during the fermentation process, the broth compositions have a significant effect on the final products. Fig.1 shows the patterns of GC/MS analysis, which compares the volatile products before and after inoculation in the T-MRS broth. Six significant peaks were detected in the T-MRS broth before inoculation, including acetic acid, glycol, lactic acid, glycerol, 4H-pyran-4-one, and 1,2,3-propanetriol. However, after 48 h of cultivation with inoculated T-MRS broth, a new peak corresponding to ethanol was detected, and the peak areas of acetic acid, glycol, lactic acid, and glycerol showed a considerable increase, whereas those of 4H-pyran-4-one and 1,2,3-propanetriol decreased and ultimately the peaks disappeared.

From the above-mentioned facts, it can be found that the composition of the volatile products in the broth had an important effect on the measurement of acid-producing abilities of the microflora.

2.2.2 Analysis of volatile products on 13 different broths

Volatile products of 13 different broths were analyzed after 48 h of cultivation using GC/MS. The results are listed in Table 3. The five products mentioned in the table were the main contributors to the improvement of the fermentation quality (Gao *et al.*, 2005). The broths were listed according to the level of lactic acid production. Lactic acid production in T-MRS, MRS, LAB, and T-juice

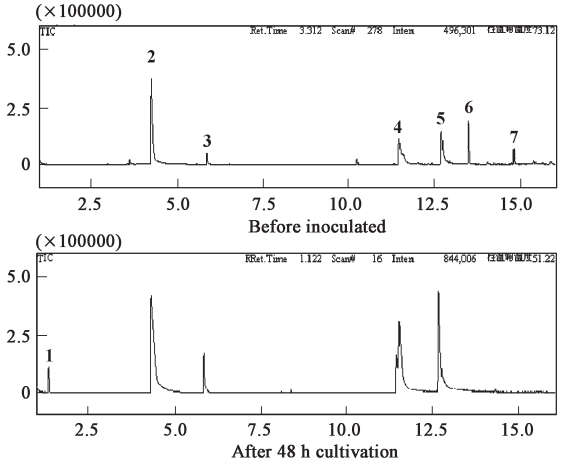

Fig. 1 Comparison of volatile products before and after inoculation for 48 h in the T-MRS broth (1) ethanol; (2) acetic acid; (3) glycol; (4) lactic acid; (5) glycerol; (6) 4H-pyran-4-one; (7) 1,2,3-propanetriol.

was significantly higher compared with the other broths (P<0.05), and the rate of glycerol production was also significantly higher in these broths (P<0.05). A significant quantity of ethanol was detected in T-MRS and MRS broths, whereas the quantity of ethanol was less or zero in other broths. Except for the SM, Zhou, and N-milk, acetic acid was detected in all the other broths, and the acetic acid production in MRS and Rogoza broths was higher compared with the other broths (P<0.05).

2.3 Microbial communities analyses

2.3.1 Analysis of DNA concentration

The DNA concentration of all the samples is shown in Fig.2. They could be divided into three groups. The highest concentration was 104.6 μg/ml in T-MRS broth. The second group included MRS, LAB, and T-juice treatments. Their concentrations were 40.3, 35.2, and 37.5 μg/ml, respectively. The DNA concentration of the third group was very low, which was close to 10 μg/ml.

Although the DNA contents of microbes were not higher, they could serve as an indicator of the counts of microbes because of their stability. So the numbers of microbes could be measured by extracting DNA from suspending liquid of a definite volume (Liu *et al.*, 2004).

Table 3 Analysis of volatile products of 13 different broths

Broth	Ethanol (g/L)	AA (g/L)	Glycol (g/L)	LA (g/L)	Glycerol (g/L)
T-MRS	1.90^{a1}	0.21^{bc}	0.69^{b}	3.46^{a}	2.17^{a}
MRS	1.22^{b}	1.47^{a}	0.68^{b}	2.97^{b}	1.61^{b}
LAB	0^{d}	0.10^{bc}	0.58^{b}	2.48^{c}	1.97^{a}
T-juice	0.42^{c}	0.18^{bc}	0.18^{cd}	2.34^{c}	1.1^{bc}
Whey	0^{d}	0.33^{bc}	1.52^{a}	1.38^{d}	2.02^{a}
GYP	0.25^{c}	0.49^{b}	0.11^{cd}	0.80^{e}	0.74^{cde}
Rogosa	0^{d}	1.74^{a}	0.56^{b}	0.6^{ef}	1.23^{b}
M-Ch	0^{d}	0.25^{bc}	0.27^{c}	0.60^{ef}	0.52^{efg}
HYA	0^{d}	0.05^{c}	0.63^{b}	0.57^{ef}	0.99^{bcd}
SM	0^{d}	0^{c}	0^{d}	0.46^{g}	0.38^{fg}
Shh	0^{d}	0.06^{c}	0^{d}	0.25^{g}	0.67^{def}
Zhou	0^{d}	0^{c}	0^{d}	0.24^{g}	0.32^{fg}
N-milk	0^{d}	0^{c}	0^{d}	0.18^{g}	0.27^{g}

[1]Within a column, means followed by different superscripts is significantly different (P<0.05); AA: acetic acid; LA: lactic acid.

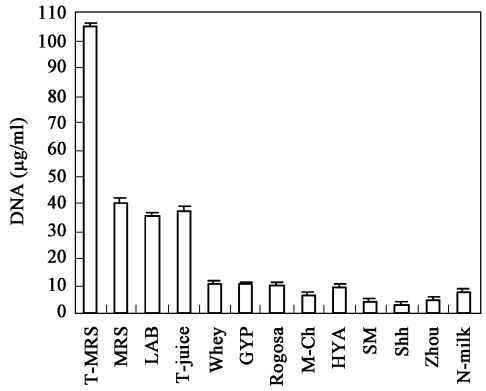

Fig. 2 Extracted DNA concentration in 13 different broths.

In this study, the DNA concentration in T-MRS was 2.5 times of that of the second group. It showed that the microbes grew more quickly in T-MRS compared with the other broths, and it also suggested that tomato juice could promote the reproduction of microbes.

2.3.2 Analysis of microbial communities by DGGE

DGGE profiles of microbial communities are shown in Fig.3. The bands on the profile were excised and amplified by PCR. After purification, the DNA fragments were sequenced and analyzed during BLAST. The phylogenetic tree was constructed (Fig.4).

The microbial species in Whey, Shh, and SM (Fig.3 columns 2, 3, 4) broths were similar to those in fermented rice straw (column 1). Besides lactic acid bacteria, there were other microbial species, such as *Enterobacter gergoviae*, *Enterobacter*, and *Pantoea endophytica* (Table 4). In future, these three broths can be used to isolate more microbial species besides lactic acid bacteria.

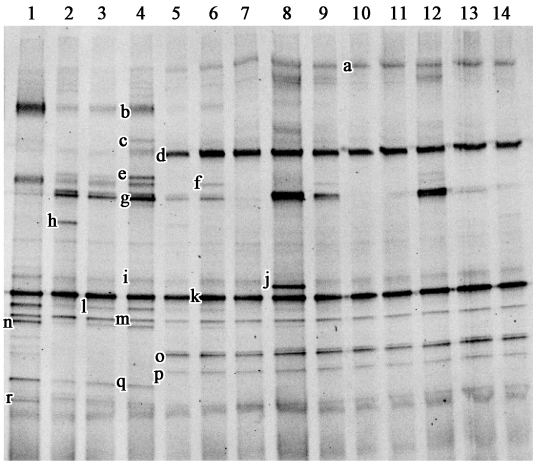

Fig. 3 DGGE profiles of microbial diversity of different broths. 1. fermented rice straw; 2. Whey; 3. Shh; 4. SM; 5. MRS; 6. HYA; 7. GYP; 8. LAB; 9. T-juice; 10. N-milk; 11. Zhou; 12. T-MRS; 13. Rogaza; 14. M-Ch.

Conservation of forage crops by ensiling is based on natural fermentation in which epiphytic LAB convert sugars into lactic acid under anaerobic conditions (McDonald *et al.*, 1991). Natural fermentation processes in crop silages, including wheat straw, alfalfa, and paddy rice silages, are dominated by species of *Lactobacillus, Pediococcus, Leuconostoc*, and *Lactococcus* (5, 29, 30). In the present study, microbes used for cultivation were from natural fermentation products of rice straw. From columns 5 to 14, these broths showed much more lactic acid bacteria (Table 4 and Fig.4). For example, although band d (*Lactobacillus plantarum*), band o (Uncultured *Lactobacillus*), and band p (Uncultured *Lactobacillus*) were not predominant in the fermented rice straw, these bands held significance in these

Table 4 Identities of DGGE bands by sequencing the excised and BLAST analysis

Band[1]	Closest relatives (accession no.)	Identity (%)	GenBank accession no.
a	*Lactobacillus paracasei* (DQ199664)	100	DQ334784
	Uncultured *Lactobacillus* (AY826745)	100	
b	*Enterobacter gergoviae* (AB004748)	97.9	DQ334785
c	*Weissella paramesenteroides* (AY190627)	91.8	DQ334786
d	*Lactobacillus plantarum* (AY851761)	100	DQ334787
e	*Brenneria quercina* (AJ223469)	87.2	DQ334788
	Uncultured bacterium (DQ068843)	87.2	
f	*Weissella paramesenteroides* (AF190630)	97.8	DQ334789
g	Uncultured Gram-positive bacterium (AB116434)	96.2	DQ334790
h	*Enterobacter* (AY579170)	99.3	DQ334791
i	*Lactobacillus bifermentans* (M58809)	86.2	DQ334792
j	*Bacillus cereus* (DQ207562)	100	DQ334793
	Bacillus thuringiensis (DQ206989)	100	
k	Uncultured gamma *proteobacterium* (DQ187905)	100	DQ334794
	Uncultured bacterium (AY985476)	100	
l	*Erwinia* (AJ971890)	97.1	DQ334795
	Enterobacter (AY579146)	97.1	
m	*Lactobacillus rhamnosus* (AF375918)	95.7	DQ334796
n	*Pantoea endophytica* (DQ123815)	96.2	DQ334797
o	Uncultured *Lactobacillus* (AF335891)	91.1	DQ334798
	Uncultured *Lactobacillus* (AY826745)		
p	Uncultured *Lactobacillus* (AF335883)	92.2	DQ334799
q	*Erwinia* (AJ971890)	97.8	DQ334800
r	*Klebsiella oxytoca* (DQ191810)	100	DQ334801

[1] Band are numbered according to Fig.3.

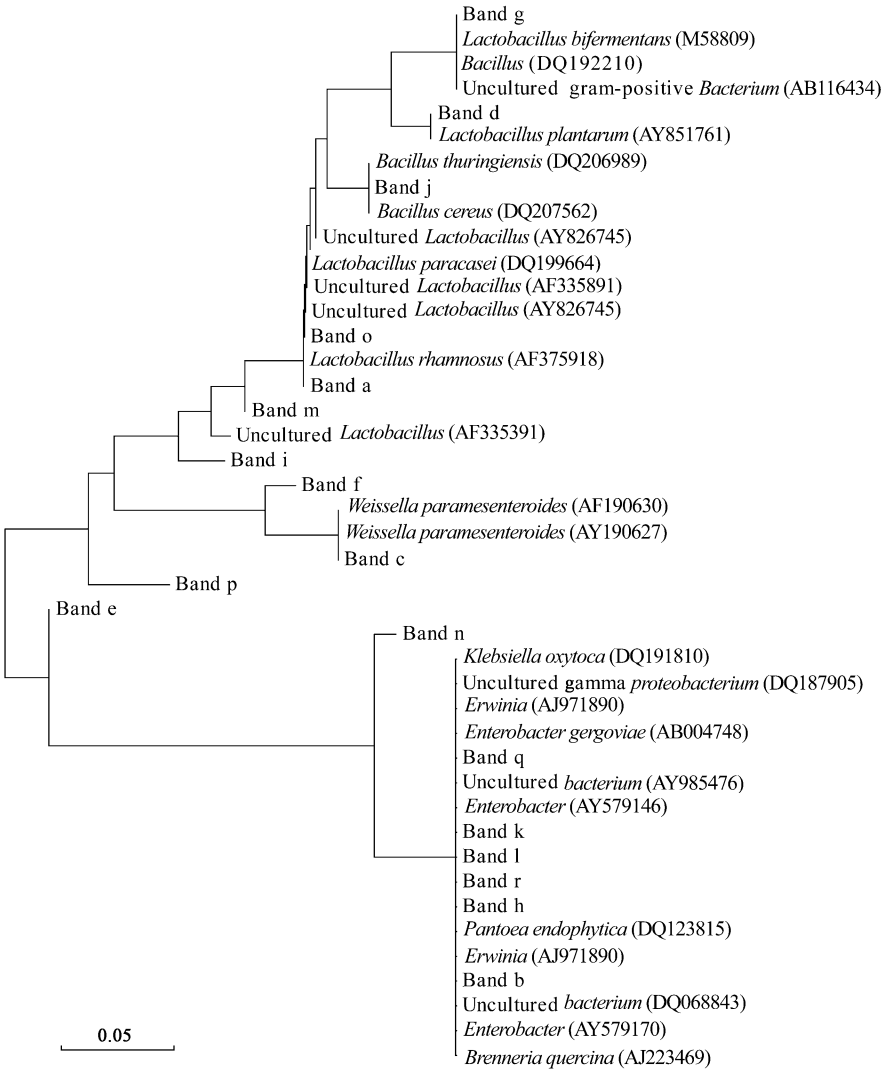

Fig. 4 Phylogenetic tree derived from partial 16S rDNA sequence. The tree was constructed by the neighbor joining method.

broths. It showed that these broths were suitable culture media for lactic acid bacteria. Heterofermentative lactic acid bacteria (band f, *Weissella paramesenteroides*) were found in HYA, T-juice, and T-MRS (columns 6, 9, and 12). And *Bacillus cereus* (band j) was found in LAB broth(column 8).

3 Conclusions

A total of 13 broths were selected to test the optimum cultivation condition for lactic acid bacteria. After 48 h of cultivation, except for the LAB, pH values in other broths could decrease to about 4.5. GC/MS analysis showed that lactic acid production in T-MRS, MRS, LAB, and T-juice was higher compared with the other broths. DNA concentration analysis showed that the counts in Tomato MRS were 2.5 times of those in other broths and suggested that tomato juice favored the reproduction of microbes. DGGE analysis showed that the number of lactic acid bacterial species in HYA, Tomato juice, and Tomato MRS was higher compared with the other broths.

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