Research on the Storage Methods of Lactic Acid Bacteria Strains Separated from DVS

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Abstract: Randomly amplified polymorphic DNA (RAPD) and morphological observation and fermentation property analysis were used to evaluate the different storage methods of three lactic acid bacteria strains separated from Directed Vat Set (DVS). **Methods:** Evaluate the genome change of the different samples from different periods during 4℃ storage, –80℃ storage and lyophilization using RAPD. Observe the morphological features by microscopy after recovered from different time of storage, then used each strain as a starter culture of fresh milk, observe the fermentation properties. **Results:** The RAPD patterns and fermentation property of the three strains all changed during 4℃ and –80℃ storage, and they all showed a good stability when stored by lyophilization. **Suggests:** This is the first time to use RAPD technique and fermentation property observation in lactic acid bacteria storage research work, RAPD appears to be an efficient method for evaluating of storage effect of lactic acid bacteria strains separated from DVS. [Nature and Science. 2004;2(4):49-53].

Keywords: DVS; lactic acid bacteria strains; storage method; fermentation properties; RAPD; morphological observation

1. Introduction

Lactic acid bacteria (LAB) are utilized in the production and preservation of various fermented foods (Jqrgen, 1999). Examples of such foods include yogurt, fermented milk, cheeses, fermented rice cake (puto), and various other foods of both plant and animal origin. Directed Vat Set (DVS) is a rapid, safety starter for the milk fermentation industry. But nobody knows what storage method is best for the LAB strains separated from DVS. The main aim of the present study was then to compare the storage methods of the LAB separated from DVS. Sonali Dixit, et al evaluated the genetic stability of plants regenerated from cryopreserved embryogenic tissues of *Dioscorea bulbifera* L. using RAPD. Finally the morphology and RAPD profiles all proved lyophilization is a proper storage method for the *Dioscorea bulbifera* embryogenic tissues (Sonali Dixit, 2003). This is the first time to compare different storage methods for the LAB strains separated from DVS using RAPD (Singh, 2004).

2. Materials and Methods

2.1 Strains: A *Streptococcus thermophillus* and two *Lactobacillus* which were separated from a DVS.

2.2 Separated Medium: M17 and MRS (Sandra, 1999) were used to separate the three strains.

2.3 Sugar Fermentation Experiment: To value the biochemical features of the three LAB strains by sugar fermentation experiment after separation. 12 different sugars were used in the experiment.

2.4 Storage Medium: The improved PY medium was used to stored in 4℃. 15% glycerin was added to the medium before it frozen in －80℃. Two improved silk milk media were used for *Streptococcus thermophillus*, *Lactobacillus* 1 and *Lactobacillus 2* lyophilization.

2.5 Morphological Features and Fermentation Properties During Storage: The strains were recovered respectively when they were stored for 1, 5, 10, 15, 30 d in 4℃. The same work was done when the strains were stored for 1, 15, 30 d in －80℃. The strains were recovered when they were stored for 90 d by lyophilization. The recovering time was recorded respectively (when the cells of the bacteria reached 10^8/ml), and the morphological features were

observed by microscopy. Each of the three LAB strains was used as the starter culture of fresh milk respectively (there were five equals for each strain), the average time was recorded when the milk changed into solid, then the average pH and qualities of the products were observed, thus the fermentation property were evaluated. All above features of the three LAB strains when just separated were tested as control.

2.6 RAPD Analysis During Storage: The three LAB strains at different time of storage were recovered respectively, then extracted their whole genome by CTAB(Kim,1990) as samples for RAPD.

PCR program was as follows: an initial denaturation consisting of 2 min at 94℃, 35 cycles consisting of 45 s at 94℃, 30 s at 38℃, and 30 s at 72℃, and then a final extension at 72℃ for 10 min. The PCR products were electrophoresed in 1.0% agarose gels, stained with ethidium bromide, and photographed.

3. Results

3.1 Sugar Fermentation Experiment (Table 1).

The sugar fermentation experiment of bacteria is shown in Table 1.

Table 1. The sugar fermentation experiment of bacteria

	Streptococcus thermophillus	*Lactobacillus 1*	*Lactobacillus 2*
Lactose	＋	＋	＋
D-Fructose	＋	＋	＋
D(+)Xylose	－	＋	＋
Sucrose	＋	－	＋
Mannitol	－	－	－
D-Sorbitol	－	－	－
Maltose	－	＋	＋
Glucose	＋	＋	＋
α , α -Trehalose	－	＋	＋
Esculin	＋	＋	＋
D-Galactose	＋	＋	＋
D-(+)-Cellobiose	＋	－	＋

'+' represented positive; '－' represented negative.

3.2 Morphological Features and Fermentation Properties During Storage

3.2.1 Morphological Features and Fermentation Properties of *Streptococcus thermophillus* During Storage (Table 2).

The Morphological features and fermentation properties of *Streptococcus thermophillus* during storage is shown in Table 2.

Table 2. Morphological features and fermentation properties of *Streptococcus thermophillus* during storage

Storage time (d)		Recovering time (h)	Morphological features	Fermentation Properties		
				Milk solidified time (h)	pH	Sense
4℃ storage	**1**	18	Cocci, singly and in short chain	5.5	4.72	Sour, milk flavor, taste common
	5	20	Cocci, in chain	6.5	4.73	Sour, not in good flavor
	10	24	Cocci, in chain	8	4.71	Sour, taste not good
	15	24	Cocci, in chain	12	4.71	Sour, taste not good
	30	24	Cocci, in chain	16	4.76	Sour, taste bad
–80℃ storage	**1**	18	Cocci, singly and in short chain	5	4.74	Sour, milk flavor, taste common
	15	24	Cocci, in chain	5.5	4.75	Sour, not in good flavor
	30	24	Cocci, in chain	8	4.73	Sour, taste not good
lyophilization	**90**	10	Cocci, singly and most in short chain	5	4.75	Sour, good flavor, taste best
Control			Cocci, in singly, pairs and short chain	5	4.73	Sour, good flavor, taste best

3.2.2 Morphological Features and Fermentation Properties of *Lactobacillus 1* During Storage (Table 3).

Morphological Features and Fermentation Properties of *Lactobacillus 1* During Storage is shown in Table 3.

Table 3. Morphological features and fermentation properties of *Lactobacillus 1* during storage

Storage time (d)		Recovering time (h)	Morphological features	Fermentation Properties		
				Milk solidified time (h)	pH	Sense
4℃ storage	1	24	Long rod, some in chain	13	4.71	Sour, flavor like milk
	5	28	Long rod, some in chain	15	4.73	Sour, not good flavor
	10	36	Long rod, in singly and chain	16	4.74	Not sour, taste bad
	15	48	Long rod, in singly and chain	18	4.76	Not sour, taste bad
	30	*				
–80℃ storage	1	24	Long rod, in singly and short chain	12.5	4.74	Sour, taste common
	15	26	Long rod, in singly and short chain	14	4.77	Sour, no good flavor
	30	26	Long rod, most in chain	16	4.76	Not sour enough
lyophilization	90	10	Long and thin rod, most singly	12.5	4.71	Sour, taste common
Control			Long and thin rod, in singly and short chain	12	4.70	Sour, taste common

**Lactobacillus 1* could not be recovered when it was store for 30 d in 4℃

3.2.3 Morphological Features and Fermentation Properties of *Lactobacillus 2* During Storage (Table 4).

The morphological Features and Fermentation Properties of *Lactobacillus 2* During Storage is shown in Table 4.

Table 4. Morphological features and fermentation properties of *Lactobacillus 2* during storage

Storage time (d)		Recovering time (h)	Morphological features	Fermentation Properties		
				Milk solidified time (h)	pH	Quality
4℃ storage,	1	24	Short rod, singly and in short chain	14	4.72	Not sour, good flavor
	5	30	Short rod, singly and in chain	15.5	4.76	Not sour, taste common
	10	38	Short rod, in short chain	16	4.74	Not sour, taste not good
	15	48	Short rod, most in chain	20	4.77	Not sour ,taste not good
	30	*				
–80℃ storage	1	24	Small rod, singly and in chain	13.5	4.72	Not sour, taste common
	15	30	Small rod, singly and in chain	14	4.73	Not sour, taste like milk
	30	30	Small rod, singly and in chain	16	4.72	Taste not good
Lyophilization	90	12	Small rod, most in chain, less singly	13	4.73	Not sour, good flavor
Control			Small rod, most in chain, some singly	13	4.72	Not sour, good flavor

**Lactobacillus 2* could not be recovered when it was store for 30d in 4℃.

3.3 RAPD Analysis When the Strains Were Stored in 4℃

The RAPD fingerprint of *Streptococcus thermophillus* changed a little since 5 d during 4℃ storage compared with 1 d (Figure 1, 1). The RAPD fingerprint of *Lactobacillus 1* changed a lot when stored for 10 d in 4℃ (Figure 1, 2). The RAPD fingerprint of *Lactobacillus 2* changed a lot from the 15 d (Figure 1, 3). Both the two *Lactobacillus* could not be recovered when they were stored for 30 d in 4 ℃.

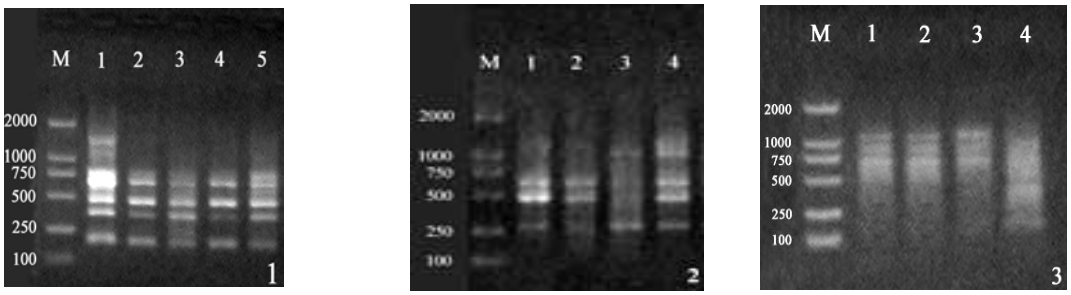

Figure 1. RAPD analysis of the strains stored for different time in 4℃. The bands were marker, stored for 1 d, stored for 5 d, stored for 10 d, stored for 15 d, stored for 30 d in proper order from left to right. (1) *Streptococcus thermophillus*. (2) *Lactobacillus 1*. (3) *Lactobacillus 2*.

3.4 RAPD Analysis When the Strains Were Stored in －80℃

The RAPD fingerprint of *Streptococcus thermophillus* changed since 15 d during －80℃ storage (Figure 2, 1). The RAPD fingerprint of *Lactobacillus 1* changed a lot when stored for 15 d, no pattern produced when stored for 30 d (Figure 2, 2). The RAPD fingerprint of *Lactobacillus 2* changed a lot during －80 ℃ storage, and no pattern produced when stored for 30 d (Figure 2, 3).

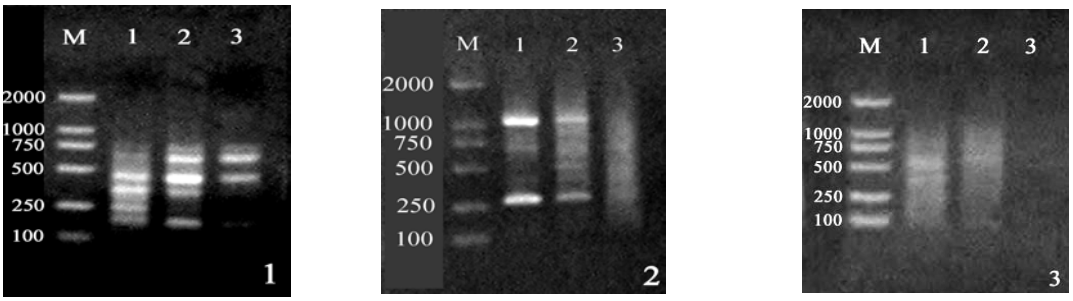

Figure 2. RAPD analysis of the strains stored for different time in －80℃. The bands were marker, stored for 1 d, stored for 15 d, stored for 30 d in proper order from left to right. (1) *Streptococcus thermophillus*. (2) *Lactobacillus 1*. (3) *Lactobacillus 2*.

3.5 RAPD Analysis When the Strains Were Stored by lyophilization

The RAPD fingerprint of the three strains did not change when they were stored by lyophilization (Figure 3).

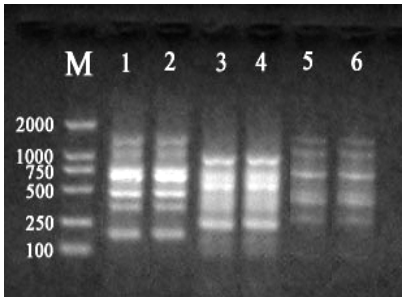

Figure 3. RAPD analysis of the strains stored by lyophilization. The bands were marker, *Streptococcus thermophillus* before lyophilization, *Streptococcus thermophillus* after lyophilization, *Lactobacillus 1* before lyophilization, *Lactobacillus 1* after lyophilization, *Lactobacillus 2* before lyophilization, *Lactobacillus 2* after lyophilization in proper order from left to right.

4. Discussion

It is known that some properties of bacteria may change when they are stored. The result of RAPD and observation of morphology and fermentation properties showed that 4℃ and －80℃ were unfit for the three strains' long time storage, after 5 days storage the fermentation properties got worse and RAPD fingerprint changed too. However, morphological features and fermentation property and RAPD fingerprint of the three LAB strains didn't change obviously after 90 d store by lyophilization.

RAPD is an easy and cost-effective profiling assay based on PCR with arbitrary primers that, by amplifying a set of DNA segments randomly distributed throughout the genome, can detect genetic polymorphisms. Nevertheless, RAPD techniques have some limitations concerning reproducibility and an uncertain homology of co-migrating fragments in gel electrophoresis. But most of these limitations can be minimized by carefully adjusting the reaction and detection conditions (Lusia C. Carvalho, 2004). In our work, we found that the RAPD fingerprint of the control group (three LAB strains) all are of certainty and reproducible.

Though LAB is wide spread in the natural environment and play an important role in several industrial and food fermentations, it is difficult to store them without function changes of these strains. So a perfect way to store LAB strains is very important to research a good starter culture for fermentation industry. The present study shows that lyophilization is the best choice for LAB strains storage through morphology observation and fermentation analysis and RAPD analysis. This is the first time to use RAPD technique as a tool to analysis LAB strains' property change when they are stored.

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