Quality examination and comparison of different brands of yogurt beverage

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Abstract: Yogurt beverage has been popular all over the world. Yogurt beverage has also been proved to be health-beneficial for people especially those with lactose intolerance since the lactic acid bacteria (LAB) strains used in yogurt fermentation are thought to be probiotic. In this research, concentrations of the living LAB of four common brands of yogurt beverage are evaluated by the dilution coating plate method and are compared with each other through one-way analysis of variance (one-way ANOVA). The result of the analysis indicates a significant difference between these samples. Gram staining and 16s rDNA sequencing are used to determine the fermentation strains of the four brands. Three of them are classified as *Streptococcus thermophilus* and one of them is classified under *Lactobacillus*, all of which are G^+.
Keywords: Yogurt, dilution coating plate method, *Streptococcus thermophilus*, *Lactobacillus*, Gram staining, one-way ANOVA, 16s rDNA sequencing.

1.Introduction

Using lactic acid bacteria (LAB) in dairy products fermentation to give them better taste and flavor is a common and ancient practice. LAB refers to a group of bacteria that ferment various carbohydrates to produce a large amount of lactic acid, including *Lactobacillus* and *Streptococcus thermophilus.* LAB utilizes the lactose through two pathways, namely Leloir pathway and Tagatose-6P pathway[1], and the end products of either pathway go through glycolysis and can be turned into lactic acid via lactic fermentation under anaerobic condition. The fermented dairy products have better taste and flavor while less lactose, so people that are lactose intolerant can uptake the nutrition in these products without suffering the symptoms of lactose intolerance like excessive flatulence, abdominal pains and diarrheas. Besides, many studies have claimed that living bacteria in yogurt can enhance lactose digestion in people with lactose intolerance[2], which emphasizes the importance of the living bacteria concentration of yogurt.

The dilution coating plate method is widely used in microorganism isolation and counting. The dilution coating plate method is also employed by the LAB evaluation in national food hygiene and safety standard[3]. Combined with the dilution coating plate method, one-way analysis of variance (one-way ANOVA) is used to demonstrate the statistical difference of the quantified quality between four brands of yogurt.

Gram staining and microscopic observation are traditional methods used to determine the characteristic of a bacteria strain. 16s rDNA sequencing is a landmark in the study of the evolution and classification of prokaryotes, based on the invention of polymerase chain reaction (PCR) and automated DNA sequencing technology[4].

2.Results

2.1 Gram staining

After isolation of the strains from the samples (marked as NO.1 to NO.4, corresponding to the four brands, detailed information is displayed in the part Methods and Materials) and cultivation on MRS culture medium plates, colonies on the same plate shared the same characteristics, so one single colony from each plate was picked up randomly for Gram staining. The results of Gram staining are shown in Fig.1, and the strain isolated from each of the four brands of yogurt was G^+.

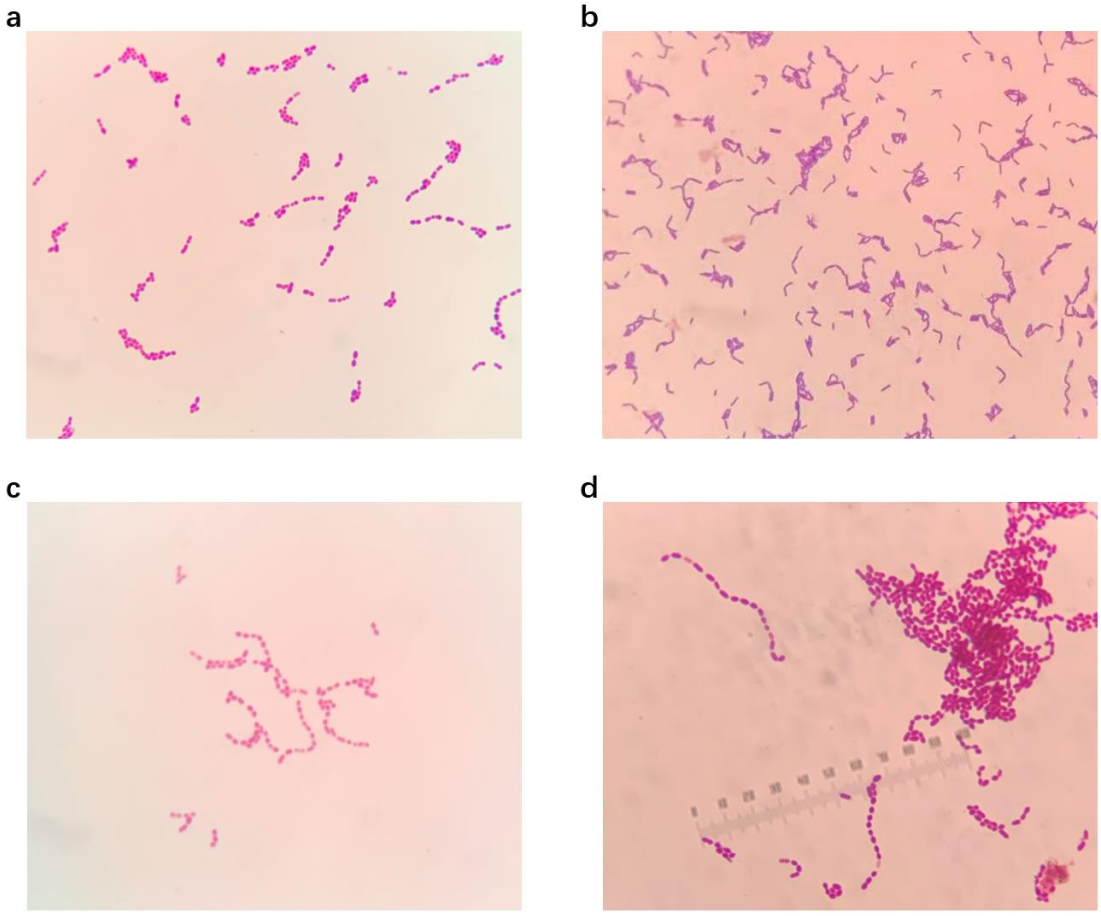


Fig. 1 Results of Gram staining. (a) The strain isolated from sample NO.1. (b) The strain isolated from sample NO.2. (c) The strain isolated from sample NO.3. (d) The strain isolated from sample NO.4.

2.2 16s rDNA sequencing

Four colonies, isolated from the four different samples, were picked up for colony PCR (Fig.2), followed by the sequencing of the PCR products.

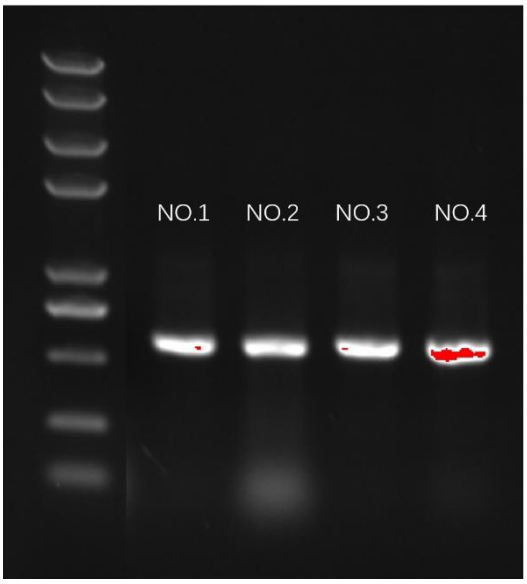


Fig. 2 Agarose gel electrophoresis of the PCR products.

After sequencing, the results were analyzed on NCBI by BLAST. The final results are shown in Table 1. The 16s rDNA sequence of the strain from Sample NO.2 showed equal similarity to several different strains under *Lactobacillus*, so this strain can be classified into *Lactobacillus*, but a specific strain can not be determined. Notably, the strains from Sample NO.3 and NO.4 showed identical 16s rDNA sequences, which means that the strains used in the fermentation of these two products are the same.

Conclusively, the strain from Sample NO.2 is *Lactobacillus* and the rest three strains are *Streptococcus thermophilus*, which is in accordance with the microscopic observation(Fig.1).

Table 1 The results of 16s rDNA sequencing.

Samples	BLAST results	Similarity
NO.1	*Streptococcus thermophilus strain CY17*	99.45%
NO.2	*Lactobacillus*	99.81%
NO.3	*Streptococcus thermophilus strain GST1*	100%
NO.4	*Streptococcus thermophilus strain GST1*	100%

2.3 Colony counting and statistical analysis

Three bottles of each of the four brands of yogurt beverage were bought for the experiments. After dilution, coating and cultivation, the colonies on each of the plates were counted and the concentration of colony-forming unit (CFU) is displayed in Table 2.

Table 2 CFU concentrations of the samples (avg ± stdev).

Samples	NO.1	NO.2	NO.3	NO.4
CFU (10^7/mL)	6.2±1.4	56.7±4.7	45.7±12.7	121.5±42.5

One-way ANOVA was then carried out by SPSS, which indicated a significant difference ($p = 0.006 < 0.01$) between these four samples. Homogeneous subsets were given out by Duncan test (n=3, confidence interval=85%) (Fig.3).

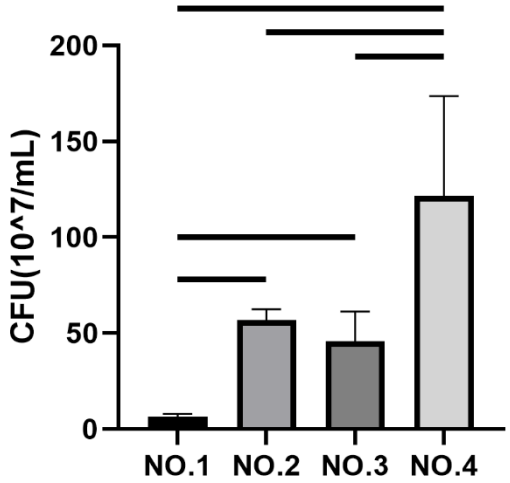


Fig. 3 CFU concentrations and comparations between each sample.
Solid line between two columns denotes significant difference.

In conclusion, Sample NO.1 has the lowest CFU concentration while Sample NO.4 has the highest CFU concentration. No significant difference in CFU concentration exists between Sample NO.2 and NO.3.

3.Discussion

In this research, both the fermentation strains and CFU concentrations were examined, which may provide a purchasing suggestion for customers.

As is shown in this work, strains used in dairy products fermentation may vary. In addition to better taste and flavor of the yogurt beverage, probiotic effects can also be conferred by the fermentation strains. Many studies have discussed the beneficial health effects of *S.thermophilus*, like prevention of chronic gastritis, alleviation of lactose intolerance and prevention of diarrhea[5]. So, it is possible to emphasize more on the health benefits for future dairy manufactures by combining different strains of LAB or genetically engineering that enhance the probiotic effects. According to the work done by García-Hernández et.al.[6], the concentration of viable *S.thermophilus* increases rapidly during consumption, but it drops after 1-2 weeks below the detection limit. Also, there is a study showing that the survival of *S.thermophilus* in the human digestive tract strongly varies from one strain to another[7]. So, if a strain of *S.thermophilus* can resident long enough in the digestive tract, long-term or even permanent lactose digestion enhancement can be expected, which would generate considerable profit and

become an elegant treatment for lactose intolerance.

In our work, the dilution coating plate method is employed to measure the CFU concentrations of four brands of yogurt beverage, which is also suggested by the national food hygiene and safety standard. However, as is shown in the results, the standard deviation can be quite big. This can be explained by our insufficiency of data, or the instability among batches of yogurt, but the dilution coating plate method may also give rise to these results since a relatively long period and protocol of experiments increases the uncertainty. In solving this problem, more repeats of the experiments can be implemented to increase the sample size and reduce the standard deviation. Besides, the turbidimetric method provides an easy measurement of cell concentrations, though death cells would also be counted through this method.

4. Materials and Methods

4.1 Materials

The yogurt beverage brands chosen for this work are displayed in Fig.4, from left to right, each of the samples is marked as NO.1 to NO.4. Three bottles of each brand were bought from a supermarket. Four bottles of different brands were used in a single repeat.

Fig. 4 Brands of yogurt beverage chosen for experiments.

The culture medium used in experiments is MRS. Ammonium oxalate crystal violet dye solution, Lugo's iodine solution, 95% alcohol, sand yellow staining solution are required for Gram staining.

Taq DNA polymerase, dNTP, PCR buffer, 16S rDNA upstream and downstream primer, TAE buffer, agarose are used in 16s rDNA PCR and agarose gel electrophoresis.

4.2 Methods

4.2.1 Dilution and coating

MRS solid medium was prepared in advance using MRS Mix according to the instruction, sterilized, and then poured into sterilized plates. Prepare 1.5 mL centrifuge tubes and add 900 μL sterile water to each tube. Mix the samples thoroughly and pour them into a sterile plate. Transfer 100 μL sample into a centrifuge containing 900 μL sterile water and mix the solution thoroughly. Before the next transferring, replace the pipette with a new one. By repeating the dilution process, we got 105-fold diluent of Sample NO.1 and 106-fold diluent of Sample NO.2, Sample NO.3 and Sample NO.4. In a single repeat of the experiment, 8 plates were coated and every two of them were coated with the same diluent (100 μL). The coated plates were stacked upside down in a sealed bag with 16 plates in each sealed bag and two bags of deoxidizing catalysts were placed in each bag. The packed plate was incubated at 37℃ for 48h, then the colony numbers were counted and the CFU values of the samples were calculated.

4.2.2 Gram staining

Take a glass slide, heat it over a light alcohol lamp, place it on a glass slide, cool it a little, and drip a drop of sterile water at the size of a mung bean in the center. After sterilization and cooling of the inoculation needle, a single colony on the plate was selected and scraped, and evenly coated in sterile water on the glass slide. Wait for the liquid to dry and the formation of the bacteria film, then pick up the slide with forceps, and let the slide pass through the flame quickly on the backside, and fix it by micro-fire. Repeat this 3-5 times. Add ammonium oxalate crystal violet dye solution to the bacterial film, dye for 1min, then wash away the dye solution. Then add Lugo's iodine solution for mordanting and wash away the iodine solution after dyeing for 1min. Then add 95% alcohol for 45s decolorization. Finally, the sand yellow staining solution was used for re-dyeing for 1min, the dye was washed away, and the remaining water droplets were absorbed dry with absorbent paper to complete the dyeing. The stained slide was placed under an oil mirror (magnification 1000 times) to observe the color and shape of the bacteria.

4.2.3 16s rDNA sequencing

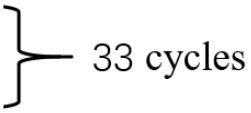
The 25 μL PCR system (components shown in Table 3)was prepared. The bacterial colonies to be tested were picked with the pipettes, and the pipettes were dipped into the PCR system and stirred gently. After the PCR reaction (program shown in Table 4), 6 μL reaction products were used for 1% agarose gel electrophoresis at 80V for 30min. The PCR products with electrophoretic strips were then sequenced.

Table 3 Components of the PCR system

Components	Volume（μL）
Buffer	2.5
dNTP	2
Taq	0.5
Primer F	1
Primer R	1
ddH2O	18
Total volume	25

Table 4 The PCR program

Process	Condition
Initial denaturation	95℃，90s
Denaturation	95℃，40s
Anneal	55℃，40s
Extension	72℃，60s
Final extension	72℃，5min
Preservation	4℃，∞



5.References

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