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The Effect of Fermentation and Storage Temperature on the Viability of *Lactoplantibacillus plantarum* subps. *plantarum* Dad-13 on Yogurt-Like Drink

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ABSTRACT: This study aims to assess the viability of the cell population during the fermentation process and storage at various temperatures. Probiotic bacteria, *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13, procured from the Center for Food and Nutrition Studies UGM, was utilized as a starter in manufacturing a yogurt-like drink. During the manufacture of the yogurt-like drink, cell counts were evaluated prior to and following a 12-hour fermentation period at temperatures of 30 °C, 37 °C, and 42 °C. The samples that have the highest viability cell (30 °C) were selected for further study and stored for 25 days at 26 °C, and for 50 days at 10 °C and 4 °C. The cell count was assessed every 5 days using MRS Media.
The result indicates that fermenting at 42 °C led to a notably lower cell growth rate of 0.2 log cycles compared to fermenting at 30 °C and 37 °C, leading to cell growth of 1.37 and 1.31 log cycles, respectively. The viability of *Lactiplantibacillus plantarum* subsp. plantarum Dad-13 in a yogurt-like drink was not significantly different after being stored for 50 days at temperatures of 10 °C and 4 °C. The decline in cell counts of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in samples stored at 10 °C and 4 °C was 0.65 and 0.34 log cycles, respectively. Conversely, at 26 °C, the viability decreased significantly, resulting in a reduction in number of 2.05 log cycles.

Keywords: *Lactiplantibacillus plantarum* subsp. *plantarum Dad-13*, lactic acid bacteria, temperature, viability

INTRODUCTION

Yogurt is the most widely produced dairy derivative product in the world. The most common starters used to produce yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Conventional yogurt cultures cannot be considered probiotics because of the lack of tolerance for acid and bile; therefore, these cultures cannot survive in the human digestive tract. (Fazilah *et al*., 2018)

In contrast to yogurt, yogurt-like drinks are milk fermentation products that use bacteria other than those in conventional yogurt cultures. One probiotic bacteria that can serve as a starter to produce yogurt-like products is *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 (Pamungkaningtyas *et al.*, 2018). This particular strain is an indigenous species of lactic acid bacteria from Indonesia isolated from Dadih, a fermented product of buffalo milk (Meidistria *et al.*, 2020). *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 has the ability to grow within a temperature range of 12-40 °C (Deshwal *et al*., 2021).

Yogurt-like drinks could have a significant impact on human health because they can provide several nutritional components and improve gut microbiota. Other benefits that can be obtained from consuming yogurt-like drinks are increased tolerance to pathogens, activation of the immune system, and enhanced absorption of lactose and essential minerals (Fadaei *et al*., 2013).

Probiotics must maintain a viability of 10^6-10^9 CFU/mL to provide a positive effect. (Meidistria *et al*, 2020). Therefore, the viability of probiotics must be maintained throughout the fermentation process, storage, and while in the digestive tract. Fermentation temperature is one of the factors that is frequently linked to the viability of probiotic cultures in yogurt-like products (Meybodi *et al*., 2020). Previous research shows that the optimum temperature for probiotic bacteria is between 37-40 °C, and using a temperature above 45 °C has a negative impact and increases the viability loss (Calinouiu *et al*., 2016). Storage temperature also has a significant role in the viability of probiotic cells; this is because the number of probiotic cells decreases during storage, and the storage temperature significantly correlates with the rate of decline in the number of probiotic cells during storage.

Previous study shows that different temperatures can affect the viability of probiotic cells, probiotic yogurt stored at 5 °C has lower viability loss than probiotic yogurt stored at 20 °C (Ferdousi *et al*., 2013). Another study also shows that *L. acidophilus* and *B. Lactis* were stored at 2, 5, and 8 °C for 20 days, L. acidophilus has the lowest viability loss when stored at 2 °C while for *B. Lactis* at 8 °C (Mortazavian *et al*., 2006). However, every bacteria has its own optimum condition that needs to be obtained to maintain its viability due to the crucial roles for the yogurt-like drink products. Thus, the purpose of this study was to understand the effect of temperature on the fermentation and storage process on the viability of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in a yogurt-like drink.

MATERIALS AND METHODS

Preparation of starter culture

Lactiplantibacillus plantarum subsp. *plantarum* Dad-13 was obtained from the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia. The *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 strain culture was isolated from curd. The single strain culture (20 mL) was inoculated at a rate of 10% (v/v) into UHT milk (180 mL) and incubated at 37 °C for 12 hours before it was ready to use as a starter culture.

Yogurt-like drink preparation

UHT milk was mixed with 3% (w/v) skimmed milk powder and then pasteurized at 85 °C for 30 minutes. The mixture was then inoculated with 10% (v/v) *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 culture and fermented at 30 °C, 37 °C, and 42 °C for 12 hours. Sampling was conducted after inoculation and fermentation to determine the growth of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13cell counts. The sample of yogurt-like with the highest cell viability was then chosen for further study, that is, the study of storage temperature. The samples were stored at 4 °C, 10 °C, and 26 °C; the samples will undergo analysis for every 5 days of storage to determine the viable cells of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13.

Enumeration of Lactiplantibacillus plantarum subsp. plantarum Dad-13

Enumeration of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 was carried out using dilution and platting methods. 0.85% NaCl solution was used to dilute the sample serially, and MRS agar with 1% $CaCO_3$ was used to enumerate the viable cells of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13. The growth of lactic acid bacteria in MRS agar was identified using CaCO3. The plates underwent incubation at 37 °C for a period of 48 hours. The enumeration of colonies was reported in CFU/mL.

Statistical analysis

The data from the experiment were examined with one-way and two-way ANOVA using SPSS 22.0 software. Duncan's multiple range test (DMRT) was utilized to assess specific differences between pairs of means with at a significance level of 5%.

RESULTS AND DISCUSSION

Change of Lactiplantibacillus plantarum subsp. plantarum Dad-13 cell counts during fermentation.

The total counts of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 cells before fermentation at 30, 37, and

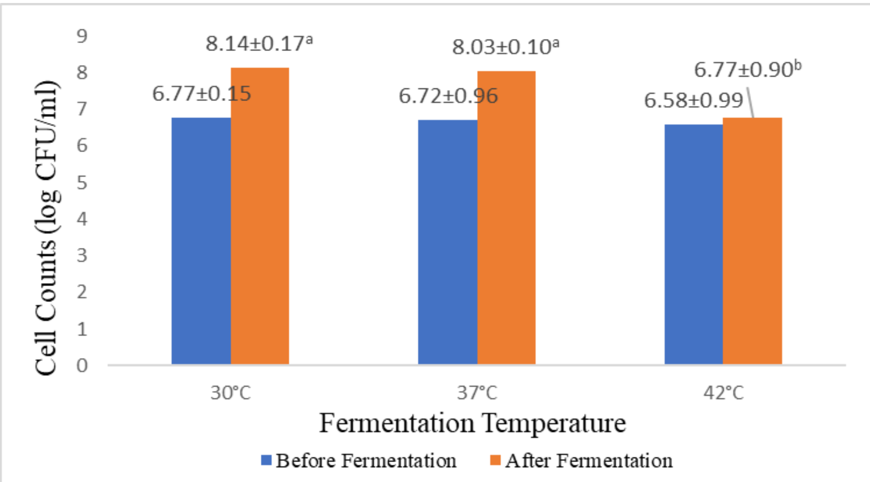

Figure 1. *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 cell counts in a yogurt-like drink before and after fermentation

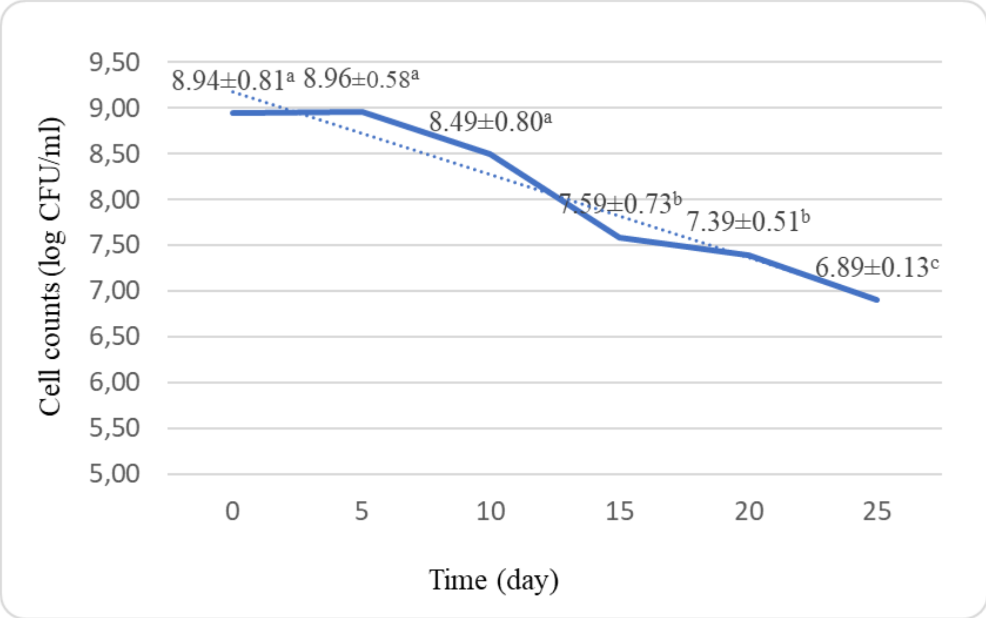

Figure 2. *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 cell counts in yogurt-like drink during storage at 26 °C

42 °C were 6.77 log CFU/mL, 6.72 log CFU/mL, and 6.58 log CFU/mL, respectively. After fermentation for 12 hours, total counts of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 ranged from 6.77-8.14 log CFU/mL. The increase in cell counts of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 after fermentation at 30, 37, and 42 °C was 1.37 log cycle, 1.31 log cycle, and 0.20 log cycle, respectively (Figure 1).

Studies showed that *L. plantarum* growth temperature ranged from 12 °C to 40 °C (Deshwal *et al*., 2021). A study conducted by Matejcekova (2016) showed that the optimum temperature for *L. plantarum* was 31.7 °C. Therefore, the fermentation temperature of conventional yogurt (42 °C), which exceeds the growth temperature range, showed a significantly lower value of cell growth compared with two other fermentation temperatures that fall within the growth temperature range (30 °C and 37 °C). The cell growth in a yogurt-like drink fermented at 30 °C has a slightly higher cell count than 37 °C due to 30 °C being near the optimum temperature for *L. plantarum* to grow. Thus, the sample of yogurt-like drink that fermented at 30 °C was chosen for the storage temperature effect study.

Viability of Lactiplantibacillus plantarum subsp. plantarum Dad-13 during storage

Storage Temperature 26 °C

The yogurt-like sample that fermented at 30 °C was selected for investigation how storage temperature due to its highest cell viability. The number of viable cells of *L. plantarum* Dad-13 in a yogurt-like drink were considerably reduced during storage at 26 °C for 25 days from 8.94 log CFU/ml to 6.90 log CFU/ml. The decrease of cell viability of *L. plantarum* Dad-13 was 2.05 log cycle (Figure 2). This could happen during storage. The starter culture involved in yogurt production would continue to carry out a metabolic process that produces lactic acid, known as post-acidification (Deshwal *et al*., 2021).

Table 1. pH measurement during storage time at 26 °C

Storage Day	pH
0	5.64
5	4.35
15	3.29
20	3.11
25	3.02

Table 1 shows the pH measurements during the storage period. Initially, on day 0, the sample's pH is 5.64, which decreases to 3.02 by day 25. The results indicate a decreasing pH over the storage period, which may be influenced by post-acidification. Post-acidification may decrease shelf life, syneresis, and sharp acid flavor development and decrease probiotics' viability and stability (Settachaimongkon *et al*., 2016). The metabolic activity of the starter in the sample is relatively high

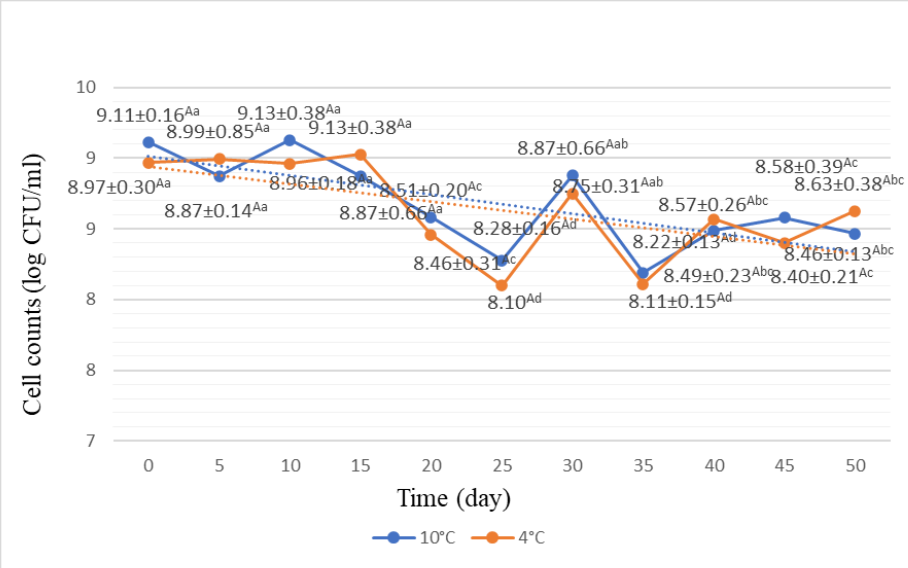

Figure 3. *Lactiplantibacillus plantarum* subsp. *plantarum Dad-13* cell counts in yogurt-like drink during storage at 10 °C and 4 °C

because the storage temperature decreased within the growth temperature range of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13. Therefore, the relatively high metabolic activity during storage resulted in a low pH of the yogurt-like drink. Furthermore, in low-pH environments, probiotics require higher energy to maintain the intracellular pH, which leads to an insufficient amount of ATP for other essential functions and may lead to cell death (Calinoiu *et al*., 2016)

Based on the study, post-acidification affected the sensory quality of samples after 15th days of storage at 26 °C. On the 15th day of storage, the yogurt-like drink had been separated and developed a sharp, sour taste and rancid flavor. A study by Lampron *et al.* (2020) showed that post-acidification could increase hydrophobic and electrostatic interaction among proteins that lead to partial restructuring of protein. Moreover, the formation of rancid flavor may occur due to oxidation of lipids contained in fermented milk products. Lipid oxidation reactions arise from oxygen and light during storage and form peroxide compounds and aldehydes (Turek *et al.*, 2018). A research conducted by Deshwal *et al.* (2021) determined that the optimum acidic condition of commercial yogurt drinks should be in the range of pH 4.0-4.4 to avoid excessive acidic taste. The decline in sensory quality resulted in the product being unfit for consumption even though the number of cells in the sample still met the requirements for probiotic food products of 10^7-10^9 CFU/mL (Rahayu & Utami, 2019). Therefore, it can be inferred that 26 °C cannot be considered an ideal storage temperature for a yogurt-like drink.

Storage Temperature 10 °C and 4 °C

The viable cells of *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in the yogurt-like drink decreased when stored at 4 °C and 10 °C for 50 days from 8.97 log CFU/mL to 8.63 log CFU/mL and 9.11 log CFU/mL to 8.47 log CFU/mL respectively. The decrease between the cell viability of these storage temperatures was 0.34 log cycle and 0.65 log cycle, respectively (Figure 3). Based on statistical analysis, there are no significant differences between the viability of probiotic cells in yogurt-like drinks stored at 4 °C and 10 °C. However, when viewed from the time during storage, the viability of probiotic cells showed significant variations with different storage durations. Throughout the storage period from day 15 to day 50, there was a notable fluctuation in the counts of probiotic cells, characterized by both increases and decreases. This fluctuation indicated that the probiotic cells were in a stationary phase of bacterial growth.

Studies showed that the stationary phase occurs when the growth rate of bacteria equals to the death rate, which stabilizes the number of bacteria within a specific range (Riadi, 2016).

A previous study conducted by Lopez *et al.* (2014) showed a similar response; there was decreasing in the viability of probiotic bacteria during 35 days of storage time at 5 °C varied from a range of 30 to 50% from the initial population. Another study also showed a similar result, there is a decreasing viability of lactic acid bacteria over a 28-day storage period at 4 °C from 10.08 to 9.98 log CFU/mL (Rossi *et al*., 2021). This situation is caused by decreased nutrient levels and the accumulation of toxic products that interfere with cell growth (Riadi, 2016). In this case, the lactose contained in the yogurt-like drink is consumed by *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13and produces metabolites in the form of lactic acid, carbon dioxide, and several other metabolites such as acetaldehyde, acetoin, and diacetyl (Hill *et al*., 2017). These metabolites will decrease the probiotic cell division rate, causing the cell growth rate to be in line with the cell death rate (Hamdiyanti, 2011).

CONCLUSION

Single culture of *L. plantarum* Dad-13 has the potential as a starter in yogurt-like drinks production. Based on the result of this research, it can be inferred that fermentation at 30 °C and 37 °C showed a significantly higher growth of *L. plantarum* Dad-13 cells compared to fermentation at 42 °C. After 50 days of storage at 10 °C and 4 °C, the cell counts of *L. plantarum* Dad-13 in yogurt-like drink ranged from 8.47-8.63 log CFU/ml, indicated that yogurt-like drink can still be categorized as probiotic product.

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