Survival of *Lactobacillus plantarum* dad 13 in probiotic cheese making

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Abstract. *Lactobacillus plantarum* Dad 13 is a group of Lactic Acid Bacteria, which was isolated from “Dadhi” (traditional fermented buffalo milk) has known as indigenous probiotic from Indonesia. Probiotics are defined as living microorganisms and have health benefits with consumption for use as a supplement of food with the amount of cell viability at least $10^8$ cells. *L. plantarum* frequently used as a starter in probiotic drink, especially in fermented milk. Milk is a valuable source of nutritional substances with the composition of water, protein, fat, sugars, mineral salts, vitamins, and enzymes for the living of microorganisms. To study the potential of *L. plantarum* Dad 13 in milk, we present an updated inventory of *L. plantarum* Dad 13 used in milk to making cheese. These we are applied *L. plantarum* Dad 13 to produce lactic acid for making curds. Combination treatment of biomass production used for cheese making that was biomass production using coconut water and MRS medium. The different combinations of a medium can influence the biomass viability of *L. plantarum* Dad 13 in cheese. The result showed that the viability of *L. plantarum* Dad 13 in cheese using two kinds of media during the production of biomass (i.e., coconut water and MRS) were almost similar during two months of storage, that was $10^3$ cfu/g. They decreased on viability after two-month storage was about 3 log cycles. The result showed that viable the cell of *L. plantarum* Dad 13 in this cheese did not agree with the criterion of a minimum of $10^6 – 10^8$ cfu/g viable cells as a probiotic product. Overall, local isolates of *L. plantarum* Dad 13 can be applied in the process of cheese making.

1. Introduction
Microbial has an essential role in the development of food products and has the potential to increase plant growth [1]. One of the microbial group, which played an important role in the food processing industry, is the lactic acid bacteria. Lactic acid bacteria are normal of the healthy gut microbial and present in numerous fermented dairy products as cheese and fermented milk [2,3]. Lactic acid bacteria are involved in various Indonesian fermented foods, and *L. plantarum* is the most common species in Indonesian fermented food, which have been studied for their characteristic properties as probiotics. Viability of *L. plantarum* Dad 13 during the process of spray drying storage at 4 °C, acid tolerance, and bile sensitivity was evaluated and showed it is potent as a probiotic [4,5]. To provide a health benefit, probiotic bacteria showed the health benefit for humans upon ingestion, where acid and bilt tolerance, antimicrobial activity, adapted to the gastrointestinal environment, and maintain high
viability at least $10^6 - 10^8$ cfu/g [4,6,7]. *Lactobacillus plantarum* can produce several natural antimicrobial substances such as bacteriocins, organic acids (mainly lactic and acetic acid), and hydrogen peroxide, which are involved in their manufacture, contribute to develop typical flavors and to improve the end of quality product [8–10].

Recently, lactic acid bacteria with probiotics properties have been given to food as potential vehicles. The nutritious and therapeutic benefits of probiotic microorganisms have been most extensively investigated in a dairy product such as milk, yogurt, and cheese [11]. Cheesemaking is based on the application of LAB to cause rapid acidification. Cheese may offer several advantages as a probiotic carrier due to consistency compared to fermented milk. Among all the dairy product and base on rate worldwide, cheese has the highest consumption because it is versatile and have been proposed as a good alternative for the delivery of probiotic bacteria to other fermented milk products [9,12,13]. There is still limited information available on the probiotic cheesemaking using *L. plantarum* Dad 13. In light of previous findings, this research was applied *L. plantarum* Dad 13 to produce lactic acid for making curds and knowing the viability of *L. plantarum* Dad 13 during the manufacturing process. The objective of this study was to study the survival of *L. plantarum* Dad 13 on probiotic cheese making and storage of the product over two months.

2. Materials and methods

2.1. Preparation of starter culture

*Lactobacillus plantarum* Dad 13 was obtained from Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Culture Studies, Gadjah Mada University, Yogyakarta, Indonesia. *L. plantarum* Dad 13 culture in MRS broth (Oxoid) was added to 20% (v/v) extract tomatoes and incubated at 37ºC for 24 h.

2.2. The production of biomass

The production of biomass with MRS broth and coconut water. Biomass, which is produced using media coconut water, was commonly added with 0.5% (w/v) glucose and 0.5% (w/v) yeast extract to encourage the growth of the culture. Before using, the two media are sterilized at 121ºC for 15 minutes. The process flow of biomass production can be seen in Figure 1. Testing the viability of *L. plantarum* Dad 13 on each stage of the process (either at the manufacturing process until the curing of cheese) made on MRS agar added with CaCO$_3$ 0.8% (v/v) with the method of spread-plate previously done some serial dilution then incubate at a temperature of 37ºC for 48 h.

![Figure 1. The process flow of biomass production.](image-url)
2.3. The procedure of cheese making
Fresh milk (cow’s milk) from Faculty Veterinary Medicine of Gadjah Mada University. Each 1 L of milk was pasteurized at 65°C for 30 min. The medium was used for making curds by adding *L. plantarum* Dad 13 and incubated at 40°C for 24 h. The curd samples were taken and stored in a cold room. The curds will take for analyzed every one week for two months storage for the viable cells of *L. plantarum* Dad 13, titratable acidity, and pH.

2.4. Enumeration of *L. plantarum* Dad 13
The population of *L. plantarum* Dad 13 was determined by the serially diluting sample in 0,85% NaCl solution and platting on MRS agar with 1% CaCO₃. After 48 h of incubation at 37°C, the colonies with the clear zone that appeared on the plates were counted and calculated as cfu/ml.

2.5. Measurement of pH and titratable acidity
The pH of the culture media was directly using a pH-meter (Eutech 510) at room temperature. Acid production was determined as titratable acidity using Sodium hydroxide titration with phenolphthalein 1% as an indicator.

3. Results and discussion
3.1. The pattern of growth and viability of *L. plantarum* Dad 13 during the production of biomass
The growth pattern of *L. plantarum* Dad 13 on the media MRS and coconut water shows almost the same growth pattern (Figure 2). At the beginning of incubation (0 h) cells density ranged from 1.3x10⁷ - 3.2x10⁷ cfu/ml. Growth of the isolates of *L. plantarum* Dad 13 on both logarithmic phase of growth media entered into a stationary phase at 2 hours until 16 hours, which ranged from 10⁸ - 10ⁱ⁰ cfu/ml. The growth curve (Figure 2) can be determined the harvest time for biomass production, which is about 16-18 hours of incubation. During the growth of pH decrease occurred in both media, which is MRS media and coconut water (Figure 3), the initial incubation at pH values ranging from 6,4 – 6,7 decreased to 3,8 – 3,9. Production of acid in curds caused a decrease in pH. These corresponded with the time of curd formation. No curd was formed with inoculum due to low acid production. In this case, the population of *L. plantarum* Dad 13 was very low, resulted in low acid production.

![Figure 2](image.png)

*Figure 2. The pattern of growth on the media MRS and coconut water, incubation at 37°C for 24 h.*
Figure 3. pH changes during growth on the media MRS and coconut water

The result testing viability of biomass production can be seen in Table 1. Based on Table 1 it can be seen that the initial cell density (hours) to biomass production is higher on MRS medium from the beginning until the end of time incubation (16 hours).

Table 1. Production of biomass L. plantarum Dad 13.

| Medium          | Begin (0 h) (cfu/ml) | The End (16 h) (cfu/ml) |
|-----------------|---------------------|------------------------|
| MRS             | 6,5x10^10           | 1,3x10^12              |
| Coconut water   | 1,1x10^10           | 9x10^11                |

Biomass result shows that medium coconut water is slightly lower compared to the production of biomass with the medium MRS; it is because the medium MRS contains complete nutrition compared to the medium of coconut water.

3.2. Viability of L. plantarum Dad 13 on The Process of Cheese Making

Results of testing the viability of cells during the process of making to the curd can be seen in (Table 2). A Total of BAL at the beginning of the product range from 2,9 x 10^4 - 2,8 x 10^6 cfu/g decrease in cell viability. BAL can be caused by several factors, including the availability of nutrients in the media, the energy in the cells, there is a buildup of acids and other metabolites results.

Table 2. Viability of L. plantarum Dad 13 during the storage (2 months).

| Cheese sample | L. plantarum Dad 13 (cfu/g) | Total of Microbial (cfu/g) |
|---------------|---------------------------|---------------------------|
|               | The end                    | Beginning                  | The end                  |
| Cheese Kontrol| 2,9x10^5                  | 1,4x10^5                  | 2,8x10^6                |
|               | 2,4x10^5                  |                          |                         |
| Cheese P_1    | 2,5x10^6                  | 1,3x10^3                  | 1,3x10^8                |
|               | 1,8x10^5                  |                          |                         |
| Cheese P_2    | 2,8x10^6                  | 1,7x10^3                  | 1,1x10^8                |
|               | 1,2x10^5                  |                          |                         |
Description: P1) cheese with BAL of biomass starter MRS. media; P2) cheese with BALES of biomass starter media coconut water.

3.3. The influence addition of L. plantarum Dad 13 against the value of pH and total acid
During the process of curing (8 weeks), the pH of cheese was decreasing from 5.40 – 4.60 (Figure 4). The values of pH on the control cheese (Non-BAL) has decreased from 4.90 - 4.74. The amount of acid secreted by L. plantarum Dad 13 in curds, will increase the acidity of cheeses (Figure 5) from 0.36% - 0.72%. Lactic acid bacteria produced the metabolism of glucose by Glycolysis Pathway through a homofermentative track and Embden Meyerhof Parnas (EMP) for the heterofermentative.

![Figure 4](image)
**Figure 4.** pH changes during the curing process cheese (8 weeks).

![Figure 5](image)
**Figure 5.** Changes of total acid-curing process cheese (8 weeks).

3.4. The influence of L. plantarum Dad 13 against the total bacteria
During curd storage, the number of bacteria has decreased on all three types of cheese, which ranged from 2.6 x 10⁶ - 1.2 x 10⁷ cfu/g (Figure 6). The decrease of the bacteria population in cheese products due to the condition of acid and low pH due to acid-organic (lactic acid) was the production by L. plantarum Dad 13. Organic acids will inhibit the growth of other bacteria that grow in the product of cheese so that the amount has decreased. On the control cheese (Non-BAL) enter week 6 - 8, the viability of total bacteria has increased, from 1.3 x 10⁸ – 2.4 x 10⁹ cfu/g.
4. Conclusion

*Lactobacillus plantarum* Dad 13 can grow well in curds for two months of storage. Based on the results of this research, it can be inferred that the viability of *L. plantarum* Dad 13 during the curds storage for two months ranged from $1.3 \times 10^3$ to $1.7 \times 10^3$ cfu/g indicated that the cheese product could not be categorized as probiotic cheese, but the isolate of *L. plantarum* Dad 13 can be used as a culture for making cheese. Single culture of *L. plantarum* Dad 13 can be used as a starter culture for making curds in cheese.

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