Effect of addition cilembu sweet potato extract (Ipomoea batatas Cilembu) as a prebiotic source for the kinetics of fermentation and lactic acid production by Lactobacillus paracasei

F Swithenia¹*, Z Bachruddin¹, A Kurniawati¹, and L M Yusiati¹

¹Faculty of Animal Science Universitas Gadjah Mada,Yogyakarta, 55281 Indonesia

*E-mail: bachruddin@ugm.ac.id and felicia.swithenia@mail.ugm.ac.id

Abstract. This study aims to determine the fermentation kinetics of Lactobacillus paracasei with the addition of Cilembu sweet potato extract as a prebiotic source. Research carried out by determining the $K_m$ constant on L. paracasei growth by analyzing the decrease in the substrate. L. paracasei grown into a defined medium with the addition of substrate with concentrations of 0.01%, 0.05%, 0.1%, and 0.2% and observed a substrate utilization at 0, 6, 18, and 24 hours with the total carbohydrate test using Anthrone. $K_m$ constant is reviewed by observing the pH levels, lactic acid production with the addition of substrates as much as 0.5 $K_m$ (1%), $K_m$ (2%) and 1.5 $K_m$ (3%). Substrate efficiency were calculated from lactic acid levels production for every concentration. Processing of research data will use a Completely Randomized Design (CRD), and if significant will continue with Duncan's Multiple Range Test (DMRT) analysis. Based on the results obtained, 1.5 $K_m$ substrate concentration shows higher consumption of substrate, lower pH, and higher lactic acid compared to 1% and 2% substrate but $K_m$ concentration shows higher substrate efficiency than 1.5 $K_m$.

Keywords: Probiotics, Prebiotics, Lactobacillus paracasei

1. Introduction

Probiotics are live microorganisms that are given as feed supplements that provide benefits by improving the balance of the intestinal microflora population. Probiotics can provide health benefits to their host [1]. Probiotics work by producing bacteriocin and short-chain organic acids (lactate, acetate, propionate). Lactic Acid Bacteria (LAB) are probiotics found in the digestive tract in animals. The LAB conditions that can be classified as probiotics are; able to survive in acidic conditions and the presence of bile, can survive during storage, and cause adverse reactions in pathogenic bacteria. [2] state that widely used probiotic microorganisms are Lactobacillus (L. bulgaricus, L. acidophilus, L. casei, L. helveticus, L. lactis, L. salivarius, L. plantarum); Bifidobacterium; Bacillus; Streptococcus; Pediococcus; Enterococcus; and yeast viz Saccharomyces cerevisiae and S. boulardii.

L. paracasei is a bacterium that can produce lactic acid from the glucose fermentation process. L. paracasei can also ferment ribose to acetic acid and lactic acid [3]. Optimum temperature L. paracasei is 30 to 37 °C [4]. L. paracasei can utilize several carbon sources for growth such as glucose, fructose, lactose, sucrose, arabinose [5], raffinose, inulin and some plant extracts [6].
The fermentation process of Lactic Acid Bacteria will be more effective with the addition of prebiotics [7]. Prebiotics are components that cannot be digested by the body but can be food for microflora in the intestine that will provide health benefits for the body. One source of prebiotics is sweet potatoes. Inayati and Putra [8] stated that this prebiotic is contained in food ingredients, one of which is contained in sweet potatoes. Raw Cilembu sweet potato varieties contain fructose 0.74-1.79%, glucose 1.60-2.67%, sucrose 0.36-1.47%, maltose 0.39-1.97%, and raffinose 0.13-0.43%.

Optimizing the use of substrates in a fermentation process is an essential factor. Adding the substrate to a fermentation process can increase the fermentation product, but if the addition of the substrate is too high, it can cause the fermentation does not run efficiently. Figueredo [9] state that the fermentation process must consider processes and mathematical analysis to achieve optimization of the product. The addition of Cilembu Sweet Potato extract with optimum concentration is expected to improve feed quality due to a good symbiotic between L. paracasei bacteria and Cilembu Sweet Potatoes.

2. Materials and Methods
This research was conducted at the Nutrition Biochemistry Laboratory, Faculty of Animal Science, Gadjah Mada University. L. paracasei comes from the collection of Nutrition Biochemistry Laboratory then rejuvenated with MRS Broth medium. Each treatment was repeated three times, and the data were analyzed with a Completely Randomized Design (CRD) in a unidirectional pattern.

2.1. Cilembu Sweet Potatoes Flour Making
Fresh Cilembu sweet potato is cleaned, peeled, and then sliced with a knife. Sweet potato slices are dried in a drying oven at 55°C for 10 hours until the sweet potato slices can be broken by hand. Sweet potato slices are then ground with blender and filtered.

2.2. Extraction
Consider carefully 5.0 grams of Cilembu Sweet Potato flour, then mixed with 90 ml of boiling water while stirring. The extract is then heated while stirring for 10 minutes. The extract solution is then filtered with filtered paper, then the solution is centrifuged, and the supernatant is taken.

2.3. Making Define Medium Fermentation Culture
Define Medium made with a mixture of mineral 1, mineral 2, yeast extract, aqua dest, and Cilembu sweet potato extract with different levels. The composition defined medium can be seen in Table 1.

| Table 1. Composition define medium |
|-----------------------------------|
| Reagent                      | Amount   |
| Mineral 1                  | 15 ml    |
| Mineral 2                  | 15 ml    |
| Yeast Extract              | 0.2 gram |
| Aquadest                   | 70 ml    |

Define the medium mixed in Erlenmeyer. Mineral 1 is made using K2HPO4,3H2O. Mineral 2 is made using K2HPO4, (NH4)2SO4, NaCl, MgSO4, and CaCl2H2O. The medium is then moved inside Hungate tube as much as 9 ml, and 1 ml of Cilembu sweet potato extract was added with concentrations of 0.01%, 0.05%, 0.1%, and 0.2%.

2.4. Substrate Utilization Analysis
L. paracasei that has been rejuvenated in MRS Broth grown into a defined medium with the addition of Cilembu sweet potato extract substrate with concentrations of 0.01%, 0.05%, 0.1%, and 0.2% and observed the substrate utilization at 0, 6, 18, and 24 hours with the total carbohydrate test using Anthrone. The total value of subsbstrate utilization at various incubation times was then changed
through logarithmic calculations to obtain Michaelis constant values from each treatment. The substrate utilization obtained was measured by connecting 1/S and 1/Vi. Equations obtained from substrate utilization curves are calculated using the following formula so that the $K_m$ constant is obtained.

$$\frac{1}{V_i} = \left(\frac{K_m}{V_m}\right) \frac{1}{S} + \frac{1}{V_m}$$

(1)

Notes:
- $V_i$ = Initial speed
- $K_m$ = Michaelis constant
- $V_{max}$ = Maximum speed
- $S$ = Substrate

2.5. Analysis of pH Levels, Lactic Acid, and Substrate Efficiency

After getting the value $K_m$ bacteria $L. \text{paracasei}$ then grow back in a liquid medium defined medium with different concentrations of Cilembu sweet potato extract which is 0,5 $K_m$ (1%), $K_m$ (2%) and 1,5 $K_m$ (3%). Samples were then tested for pH and lactic acid levels produced at the 12th hour with three replications. Substrate efficiency were calculated from lactic acid levels produced for every concentration with assumption the substrate were used to produce lactic acid.

3. Result and Discussion

Microbial growth is the main thing that must be studied in the fermentation process. One way to find out microbial growth is by knowing the substrate decrease curve or substrate consumption in the fermentation process. Bachruddin [10] states that the rate at which the substrate changes to product compounds depends on the amount of substrate content undergoing unit time transitions. Okpokwasili and Nweke [11] state that the speed of microbial growth and substrate concentration is essential in biotechnology. With the substrate drop curve, logarithmic phase information can be obtained from microbial growth. This curve determines to observe the decrease in the substrate with time variables. The substrate utilization curve can be seen in Figure 1.

Figure 1 shows that Cilembu sweet potato extract concentration 0,2% showed the highest substrate reduction rate compared to the substrate reduction rate with a concentration of 0,01; 0,05; and 0,1%. The lowest rate of substrate reduction was the addition of cilembu sweet potato extract with a concentration of 0,01%. This indicates that the higher the amount of substrate, the higher the rate of substrate reduction. Bachruddin [10] states that the rate at which the substrate changes to product
compounds depends on the amount of substrate content undergoing unit time transitions. The Vi obtained from the substrate decrease curve is then made into a 1/Vi curve against 1/Substrate. The Km and Vmaxx L. paracasei curve can be seen in Figure 2.

The graph in Figure 2, the Km constant is obtained L. paracasei to the Cilembu sweet potato substrate of 2 gram/100 ml with Vmax amounting to 0,1405 gram/100ml/hour. Based on these results it can be seen that bacteria L. paracasei requires 2 grams of Cilembu sweet potato in 100 ml of medium for optimal bacterial growth and fermentation to run efficiently.

Table 2. Result of pH, lactic acid production, dan substrate efficiency by L. paracasei

| Treatment | pH     | Lactic Acid | Substrate Efficiency |
|-----------|--------|-------------|----------------------|
| P1        | 4.27±0.03<sup>c</sup> | 0.89±0.39<sup>a</sup> | 8.934±0.39<sup>b</sup> |
| P2        | 4.07±0.98<sup>b</sup> | 1.23±0.98<sup>b</sup> | 6.150±0.492<sup>a</sup> |
| P3        | 3.95±0.31<sup>a</sup> | 1.56±0.55<sup>c</sup> | 5.197±0.184<sup>a</sup> |

Note: P1 : Levels of substrate 0.5 Km (1%)  
P2 : Levels of substrate 1 Km (2%)  
P3 : Levels of substrate 1.5 Km (3%)  
Different superscripts on the pH and lactic acid line show differences (P <0.05)  
Different superscripts on the substrate efficiency line show differences (P <0.01)

The results of the pH test at the end of the fermentation of bacteria L. paracasei Table 1 shows that the fermentation of P1 produced the highest pH of the solution. The P3 fermentation is resulting in the lowest solution of pH. Lactic acid production in the fermentation of P1 produces the lowest lactic acid. P3 fermentation produces the highest lactic acid. The substrate concentration of each treatment is by the levels of lactic acid and the pH value produced in each sample. High substrate concentration causes high production of lactic acid and low pH. It indicates that the substrate is an essential factor for microbial growth. If the substrate given is not suitable or the amount is small, the fermentation
process will run slowly and not optimal. Rahmayetti [12] states that the success of the fermentation process was influenced by the type and concentration of the substrate, the pH, and the concentration of bacteria. Afriani [13] states that the success factor of fermentation is very much determined from the substrate. Microbes need energy from carbohydrates to be able to grow and easily adapt to their environment.

The value of the substrate efficiency at a concentration of 0.5 \( K_m \) namely 8.934%. The results obtained were lower than [14] which states that lactic acid bacteria can produce 0.83 grams of lactic acid per 1 gram of fermented starch. The value of the substrate efficiency at a concentration of 0.5 \( K_m \) is the highest score. This can be due to the substrate in the form of Cilembu sweet potato extract with a concentration of 0.5 \( K_m \) provided can be utilized by \( L. \) paracasei compared to the concentration of \( K_m \) and 1.5 \( K_m \) [15] stated that the consumption of sugar \( L. \) paracasei decreases with increasing sugar concentration given. The higher the sugar concentration, it can inhibit the production of biomass and fermented products. Cilembu sweet potato extract which is used as a substrate is not a pure carbohydrate component as a prebiotic source. The substrate with a concentration of 0.5 \( K_m \) contains inhibitory compounds lower than the concentration of \( K_m \) and 1.5 \( K_m \). [16] stated that the use of glucose as a carbon source in fermentation \( L. \) paracasei more efficient in the production of lactic acid compared to fructose and sucrose. High sucrose can increase the viscosity of the solution.

4. Conclusion
The substrate content of 2% or equivalent to the \( K_m \) constant obtained based on the results of the analysis has not produced an optimal product, however the efficiency of substrate utilisation is better at \( K_m \) than 1.5 \( K_m \). So there needs a retest to find out the levels or purified of Cilembu sweet potato extract as an appropriate substrate as a prebiotic for \( L. \) paracasei bacterial fermentation run optimally.

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