Antimicrobial Activity of Set Yoghurt Probiotic from Milk as a Functional Food

C Hartati¹, R L Balia¹, L Suryaningsih¹, E Wulandari¹ and W S Putranto¹

¹Department of Animal Husbandry Technology, Faculty of Animal Husbandry, Universitas Padjadjaran, Bandung, Indonesia
E-mail : wendrysp@yahoo.co.id

Abstract. Milk is generally a product that is quickly damaged by microbes due to the complete and balanced nutritional content of milk so that microbes are very easy to grow. Preservation technology is needed to increase durability of milk and one of them by fermentation using Lactic Acid Bacteria (LAB). Seyoghurt products with mung bean extract supplementation and BAL starter used with Lactobacillus casei ALGHCH 212 combination can effectively inhibit pathogen bacteria growth (E coli, Listeria, Pseudomonas, S aureus) in 24 hours incubation compared with control of antibiotic ampicillin with a concentration of 31.25 mg/ml.

1. Introduction
Milk is a potential livestock commodity developed into food products of high economic value. Innovation in producing new dairy products is needed as an effort to increase food diversification and support national food security. Livestock commodities are rapidly damaged primarily by microbes, as well as with milk. Fermentation by utilizing lactic acid bacteria is an attempt to preserve and produce a processed product known as yoghurt.

Yogurt with raw material of cow's milk is quite popular in the community. Based on different methods of manufacture, yogurt type is divided into two namely, Stirred Yogurt is Yogurt which fermentation process is done on a large tank / container and after incubation then the product is packed in small packaging, thus allowing the coagulum is broken or broken before cooling and packaging finished and Set Yogurt is yogurt which at the time of incubation / fermentation of milk is in the small packaging and coagulum characteristics are not changed. Yogurt that spread on the market today is yogurt type Stirred Yogurt, while the Yogurt Set is still rarely developed and people are less familiar with Yogurt Set when compared to Stirred Yogurt, Set Yogurt has better consistency. As an effort to diversify food product, a combination of milk and vegetable extract in yoghurt is made.

Mung beans (Vigna radiata L) have balanced nutrients and are rich in antioxidants [1]. Mung bean extract (Mung Bean Milk) fermented with Lactobacillus plantarum B1-6 potentially developed as a functional food [2]. Use of 30% green beans juice in whey fermented tofu with starter Lactic Acid Bacteria provides best lowering lactose levels, increasing acidity and raising total solids [3].

This research is expected to produce a new product of probiotic fermentation beverage which is good for health. It is expected to find a combination of milk and vegetable extracts and the right starter formulation to produce yoghurt products that meet the standards as a probiotic drink.

2. Materials and Method

2.1. Growth curve of Lactic Acid Bacteria
The observation of BAL growth curve is Streptococcus thermophilus (ST), Lactobacillus bulgaricus (LB), Lactobacillus acidophilus (LA), Lactobacillus casei ALGHCH.2.12 (LC ALG 2.12) on MRS
Broth media and incubation temperature 370 C, non shaking and bacth system. Sampling dilakuan every 5 hours and measured absorbance at 600 nm wavelength.

2.2. Making Mung bean Extract
The cleansed mungbeans are soaked in hot water for 8 hours, then boiling and adding water (1: 6). Furthermore, screening and extract of green beans was obtained.

2.3. Specific Growth Rate
Observation on logarithmic phase of BAL isolate on MRSB media with Mung bean extract supplementation with ratio (1: 1).

2.4. Setyoghurt of Mung bean Extract and Starter Combination
The treatment includes the following combinations:

| Groups | Combinations of starter |
|--------|------------------------|
| P1     | P2                     |
| Ys     | YsP1                   |
| KH     | KHP1                   |

Ys = Milk (50 ml)
KH = Milk : Mung bean extract (1:1) (25 ml : 25 ml)
P1 = 5% (2.5 ml) (ST : LB : LA ) (1:1:1)
P2 = 5% (2.5 ml) (ST:LB:LA) (1:1:1) = 1.875 ml + LC ALG 2.12 0.625 ml
P3 = 5% (2.5 ml) (ST:LB:LA) (1:1:1) = 1.875 ml + BF 0.625 ml

2.5. Bacterial Resistance Testing with Agar Diffusion
Testing of antimicrobial activity using well diffusion method against some pathogenic bacteria and BAL. The culture of the first test bacterium was cultured on Nutrient Broth (NB) 10 ml overnight at 37 °C, then calculated the number of bacterial cells up to 107 per ml. Further inoculated as much as 0.1% into 20 ml of Nutrient Agar medium, was carried out to homogeneous so that the test bacteria was chalked flat, then poured into sterile petri dish, and left to solid then made well with diameter 6 mm (5 wells per cup). The sample set of 50 µl yogurt was fed into the well then incubated at 37 °C for 24 hours. Observations were made by measuring the zone of inhibition based on the diameter of the clear area formed around the well.

3. Result and Discussion
Observation of the growth curve through the absorbance value of BAL isolate culture on MRSB at 600 nm wavelength. In MRSB media all BAL isolates showed logarithmic phase at 5 th to 20 th incubation period at 37 °C. Thus, logarithmic phase information is used as reference in conducting analysis of fermentation efficiency by considering the value of Specific Growth Rate (µ) of each isolate of BAL. The increase in value (µ) also shows an increase in fermentation efficiency as a result of extrinsic factor manipulation such as the use of prebiotics. The growth curve begins with the initial phase (lag) which is the period of microbial adjustment. At that phase enzyme synthesis occurs by cells used for metabolism. After the initial phase is complete, new cellular reproduction begins. Cellular concentration increases, initially slowly and then increasingly until one day the rate of cellular growth or reproduction reaches the maximum and logarithmic or exponential growth occurs. Furthermore, after certain substrate or compound is required for bacterial growth in the culture
medium approaching and there is accumulation of inhibiting products, there is a decrease of bacterial growth rate. The decline phase is characterized by a decrease in the number of living cells (viable) in the media due to mortality (mortality) [4].

![Growth rate curve of](image)

Figure 1. Growth rate curve of *Streptococcus thermophilus* (ST), *Lactobacillus bulgaricus* (LB), *Lactobacillus acidophilus* (LA), *Lactobacillus casei* ALG.2.12 (LC ALG 2.12), in MRS Broth at 37 °C, non shaking dan bacth system.

3.1. Mung bean Extract Supplementation

At 37 °C incubation the entire isolate undergoes a logarithmic phase at 5 to 20 hours, so we can analyze the specific growth rate at that time span with the value (µ) = hour⁻¹ as follows:

| Isolat Bakteri Asam Laktat | Specific growth rate (µ) = hours⁻¹ |
|---------------------------|-----------------------------------|
| *Streptococcus thermophilus* (ST) | 0.1503 (MRS) - (MRS + Mung bean extract) |
| *Lactobacillus bulgaricus* (LB) | 0.2415 (MRS) 0.2621 (MRS + Mung bean extract) |
| *Lactobacillus acidophilus* (LA), | 0.1230 (MRS) 0.1547 (MRS + Mung bean extract) |
| *Lactobacillus casei* ALG.2.12 (LC ALG 2.12) | 0.1382 (MRS) 0.1641 (MRS + Mung bean extract) |

The use of green bean extract was able to increase *Lactobacillus bulgaricus* (LB) Specific Growth Rate, *Lactobacillus acidophilus* (LA), *Lactobacillus casei* ALG.2.12 (LC ALG 2.12).

3.2. Set Yoghurt Mung bean Extract

One of the milk processing technology is by fermentation using lactic acid bacteria and the product we know is yoghurt. The fermentation process with starter of lactic acid bacteria causes the milk to coagulate until it is obtained by its acidity, distinctive odor and flavor, with visions of viscous liquid until semi-solid. Dry matter content of yogurt at least 12% [5] and pH yoghurt 4.4-4.5 [6]. The method of making yoghurt in the absence of stirring and the addition of flavor and flavor at the end of the
yogurt making process is known as the Set yoghurt. The yoghurt coagulum characteristics were unchanged, since the Set yoghurt were not stirred after incubation such as Stirred Yoghurt, but incubation after packaging in a sealed packet that could be consumed immediately [6].

Figure 2. Set yoghurt extract green beans with various starter formulations BAL

3.3. Inhibitions of Set Yoghurt Green Bean Extract Againsts Pathogenic Bacteria
The set of green bean yoghurt extract that has been produced by bioassay to know its resistance to pathogen bacteria.

Figure 3. Inhibition set yoghurt extract green beans on growth pathogenetic bacteria with agar diffusion method at 37 °C incubation during 24 hours

Lactic acid bacteria in the fermentation of food in addition to providing a distinctive flavor, this bacteria also extend durability due to its ability to produce metabolite products that can inhibit the growth of bacterial decay and bacterial pathogens. The antimicrobial properties possessed by lactic acid bacteria are due to their suitable conditions with the available nutrients, which can lead to competition with other bacteria especially pathogenic bacteria [7]. Lactic acid bacteria (BAL) produce lactic acid as the end of sugar metabolism (carbohydrate). Lactic acid produced in such a way will decrease the pH value of its growth environment and cause a sour taste. BAL ability to produce proteolytic enzymes around cell walls, cytoplasmic membranes, or inside cells and it is known that Lactobacillus acidophilus has the ability to produce extracellular proteases [8]. The antimicrobial properties possessed by lactic acid bacteria are due to their suitable conditions with the available nutrients, which can lead to competition with other bacteria especially pathogenic bacteria [7].
Table 2. Diameter of the resistor zone set of yoghurt extract of green beans compare with antibiotic control.

| Sample  | Inhibitions (mm) at incubation 24 hours |  |  |  |
|---------|--------------------------------------|---|---|---|
|         | E coli | Listeria | Pseudomonas | S aureus |
| YsP1    | 14.5   | 15.4     | 11.2        | 15.3     |
| YsP2    | 14.7   | 15.2     | 12.4        | 13       |
| YsP3    | 15.8   | 17.9     | 11.7        | 14.8     |
| KHP1    | 12.9   | 13.1     | 13.9        | 15.1     |
| KHP2    | 12.8   | 15.1     | 14.7        | 12.9     |
| KHP3    | 12     | 16.3     | 14.6        | 14.7     |
| Control Antibiotic 31.25 mg/ml |          |          | all antibiotic controls are largely unable to inhibit the growth of tested bacteria |

4. Conclusions

The use of mung bean extract was able to increase *Lactobacillus bulgaricus* (LB) *Lactobacillus acidophilus* (LA), *Lactobacillus casei* ALG.2.12 (LC ALG 2.12) spesific growth rate. Set yoghurt with mung bean supplementation and using Lactic Acid Bacteria starter with *Lactobacillus casei* ALG212 combination (LC ALG 2.12) can effectively inhibit pathogen bacteria growth (*E coli, Listeria, Pseudomonas, S aureus*) in 24 hours incubation compared with control antibiotic ampicillin with a concentration of 31.25 mg / ml.

Acknowledgements

We would like to thank Rector University of Padjadjaran, who has funded this research through Academic Leadership Grant (ALG) 2017.

References

[1] Shi Z, Yao Y, Zhu Y, Ren G 2016 Nutritional composition and antioxidant activity of twenty mung bean cultivars in China *The Crop Journal* **4**: 398-406.
[2] Wu H, Rui X, Li W, Chen X, Jiang M, Dong M 2015 Mung bean (*Vigna radiata*) as probiotic food through fermentation with *Lactobacillus plantarum* B1-6. *LWT*.
[3] Nawangsari D N, Legowo A M, Mulyani S 2012 Kadar laktosa, keasaman dan total bahan padat whey fermentasi dengan penambahan jus kangkang hijau *Jurnal Aplikasi Teknologi Pangan* **1(1)**.
[4] Mangunwidjaja D, Suryani A 1994 Teknologi Bioproses *Penebar Swadaya* Jakarta.
[5] Standar Nasional Indonesia (SNI) 1992 SNI 01-2981-1992 Yogurt *Pusat Standarisasi Industri* Jakarta.
[6] Bylund G 1995 Tetra Pack Processing Systems *Dairy Processing Handbook* AB Lund. Sweden pp. 383-393.
[7] Codex STAN 2003 Codex Standard For Fermented Milk (Codex STAN 243-2003) Codex Alimentarius.

[8] Tamime A Y, Deeth H C 1980 Yoghurt. Technology and Biochemistry J. Food Protection 43(12): 939-977.