**Averrhoa bilimbi** fruit filtrate as the source and growth medium for lactic acid bacteria

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**Abstract.** The study aimed to investigate the potential of *Averrhoa bilimbi* fruit filtrate as the source and growth medium for lactic acid bacteria (LAB). The ripe *A. bilimbi* fruit was thoroughly washed, drained, blended and filtered using cheesecloth. The fresh filtrate was stored in the freezer and used as control (T0). Another fruit filtrate was placed in an anaerobic jar and spontaneously incubated at room temperature (±25°C) for 4 days (T1). The rest of the filtrate was put in an anaerobic jar, added (1 g/100 mL) with shrimp paste (as a source of LAB) and incubated at room temperature for 4 days (T2). The number of LAB, pH values and antioxidant activity of the fruit filtrates were then determined. Results showed that fresh filtrate of *A. bilimbi* fruit contained substantial numbers of LAB (6.69 log cfu/mL), and after 4 days of spontaneous anaerobic incubation the numbers of LAB increased significantly (25.2 log cfu/mL). The addition of shrimp paste further increased the LAB populations (30.4 log cfu/mL) in the incubated fruit filtrate. pH values were substantially lower in T2 than that in T0 and T1. The antioxidant activity (percentage of inhibition of 1,1-Diphenyl-2-picryl Hidrazil [DPPH]) did not differ among the groups. In conclusion, the ripe *A. bilimbi* fruit could be a good source and growth medium for LAB.

1. Introduction

The use of lactic acid bacteria (LAB) as probiotics in poultry production has commonly been practiced. To be commercially produced, the LAB-based probiotics must be grown or cultured in the specific media with particular nutrients requirements. The latter need may therefore increase the production cost for LAB-based probiotics. Recent study showed that acid fruits naturally contain LAB that can exhibit probiotic activity [1]. *A. bilimbi* (belonging to the Oxalidaceae family) is one of the acid fruits that are easily found in tropical countries such as Indonesia. The fruit is rich in phenolic compounds, vitamin C and tannin [2]. It also contains amino acid and glucose, which may be used as the substrate for LAB growth [3]. Apart from these facts, the use of *A. bilimbi* fruit as the source and growth medium for LAB has never been elucidated.

Shrimp paste has widely been known as a traditional food seasoning with special flavor. This is produced from the spontaneous fermentation of shrimp. As the product of fermentation, shrimp paste is rich in LAB that can serve as probiotic [4]. Maeda et al. [5] previously reported that there were some LAB species isolated from shrimp paste, including *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactococcus garvieae* and *Vagococcus fluvialis*. Among these LAB species, *L. plantarum* was the most dominant LAB species [4]. In this study, shrimp paste was added to the *A. bilimbi* fruit filtrate as the source of LAB. The present study aimed to investigate the potential of *A. bilimbi* fruit filtrate as the source and growth medium for LAB.

2. Methodology

The ripe *A. bilimbi* fruit was harvested from the gardens around the campus. The ripe fruit was characterized by the yellowish colour and a bit of tender texture. The collected fruit was thoroughly washed with running water and drained. The fruit was then blended using an electric blender and filtered using cheesecloth to produce fruit filtrate. The fresh filtrate was stored in the freezer and used as control (T0). Another fruit filtrate was placed in an anaerobic jar and spontaneously incubated at room temperature (±25°C) for 4 days (T1). The rest of the filtrate was put in an anaerobic jar, added (1 g/100 mL) with shrimp paste (as a source of LAB) and incubated at room temperature for 4 days (T2). The...
number of LAB, pH values and antioxidant activity of the filtrates were then determined. The experiment was conducted in four replicates.

The numbers of LAB in the fruit filtrate was counted following the total plate count procedure on de Man, Rogosa and Sharpe agar media (MRS; Merck KGaA, Darmstadt, Germany). The counting was done after the plate was anaerobically incubated at 38°C for 2 days. The pH values of fruit filtrate was conducted using a digital pH meter (Eco Test pH 1). Antioxidant activity was conducted according to a DPPH free radical neutralizing assay [6]. The fruit filtrate (0.5 mL) was diluted in 1.1-Diphenyl-2-picryl Hidrazil (DPPH) solution (3 mL). The absorbance of the sample solution and the blank were measured after 30 min at room temperature. The change in the DPPH colour was observed on a spectrophotometer at 517 nm. The inhibition of free radicals by DPPH (in %) was determined based on the equation: 100 × (A\text{blank} − A\text{sample})/A\text{blank}.

Data were subjected to analysis of variance (ANOVA) with statistical software of SPPS (IBM SPSS Statistic version 23). Duncan multiple range test (SPSS) was then performed when the effect of treatments was significant at the level of 5%.

3. Results and Discussion

Data on the numbers of LAB, pH and antioxidant activity of A. bilimbi fruit filtrate are presented in Table 1. It appeared that LAB populations were detected in the fresh filtrate of A. bilimbi fruit in substantial amounts, confirming that fresh A. bilimbi fruit could be a good source of LAB. This finding was in agreement with Rodriguez et al. [1] who previously showed that acid fruits were good sources for probiotic-based LAB. However, we did not assess the probiotic activity of LAB from A. bilimbi fruit in the present study. Interesting finding was also seen in this study, at which the numbers of LAB increased significantly after anaerobic incubation for 4 days at room temperature. Also, the addition of shrimp paste further increased LAB populations following 4 days incubation at room temperature. This was in accordance with Kobayasi et al. [7] reporting that in general the LAB colonies in the Indonesian shrimp paste ranged from 4 to 6 log cfu/g. Together, the data may confirm that A. bilimbi fruit filtrate was able to support the growth of LAB, regardless of the origin of the LAB. The contents of glucose and amino acids as well as other nutrient components in the fruit [2,3] seemed to fulfill the need for substrates for LAB growth.

Data obtained in this study showed that the pH values of fresh A. bilimbi fruit filtrate was about 1.45, which was within the normal range for the pH values of A. bilimbi fruit (0.9-1.5) [3]. In this study, pH values were substantially lower in T2 than that in T0 and T1. Indeed, the pH values decreased with the increased numbers of LAB in the fruit filtrate. Narendranath and Power [8] reported that the growth of LAB was associated with the production of acid products, which eventually reduced the pH of the medium. The fresh A. bilimbi fruit filtrate showed high antioxidant activity as indicated by the percentage inhibition of DPPH, which was higher than 50%. Our present result was actually in agreement with Kolar et al. [9] documenting that the DPPH inhibition of A. bilimbi fruit extract was 50.72-58.53%. In this study, the increased numbers of LAB and decreased pH values had no impact on the antioxidant activity of A. bilimbi fruit filtrate. This was in contrast to Livinska et al. [10] showing the antioxidant activity in some LAB strains isolated from dairy products and fermented fruits, and Ding et al. [11] showing the antioxidant activity of Lactobacillus delbrueckii F17. However, it should be noted that different species and strains of LAB may show different antioxidant activities.

| Items                           | T0      | T1      | T2      | SEM     | P value |
|---------------------------------|---------|---------|---------|---------|---------|
| LAB numbers (log cfu/mL)        | 6.69\(a\) | 25.2\(b\) | 30.4\(a\) | 3.08\(\) | <0.01   |
| pH values                       | 1.45\(a\) | 1.43\(a\) | 1.30\(b\) | 0.03     | 0.02    |
| Antioxidant activity (DPPH, %)  | 65.2\(\) | 67.8\(\) | 64.9\(\) | 1.08\(\) | 0.53    |

\(a,b\): Values with different letters within the same row are notably different (P<0.05)

T0: fresh A. bilimbi fruit filtrate stored in freezer (control). T1: fruit filtrate placed in anaerobic jar and spontaneously incubated at room temperature for 4 days. T2: fruit filtrate placed in anaerobic jar, added with shrimp paste and incubated at room temperature for 4 days. SEM: standard of the means.
4. Conclusion
The ripe *A. bilimbi* fruit could be a good source and growth medium for LAB.

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References
[1] Rodríguez L G R. *et al* 2019 *Front. Microbiol.* **10** 1091
[2] Patil AG *et al* 2013 *J. Pharm. Res.* **6** 145
[3] Sugiharto S 2020 *J. Adv. Vet. Res.* **10** 179
[4] Amalia U *et al* 2018 *IOP Conf. Ser. Earth Environ. Sci.* **116** 012049
[5] Maeda M *et al* 2014 *Mar. Biotechnol.* **16** 181
[6] Sohaib M *et al* 2012 *Lipids Health Dis.* **11** 1
[7] Kobayashi T *et al* 2003 *J. Gen. Appl. Microbiol.* **49** 279
[8] Narendranath N V and Power R 2005 *Appl. Environ. Microbiol.* **71** 2239
[9] Kolar F R *et al* 2011 *Afr. J. Pharm. Pharmacol.* **5** 2067
[10] Livinska O *et al* 2016 *AIMS Microbiol.* **2** 447
[11] Ding W *et al* 2017 *J. Funct. Foods.* **35** 481