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Prebiotic Activity of Ambon Banana (*Musa acuminata* (AAA Group) 'Ambon') Peel Starch Against *Lactobacillus. acidophilus* and *Escherichia coli* In Vitro

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ABSTRACT

Prebiotics are compounds with the ability to specifically enhance the population of advantageous bacteria in the gastrointestinal tract. Starch is a polysaccharide which has prebiotic activity. Agricultural waste like banana peel contains prebiotic polysaccharides including starch. The prebiotic effect of starch from many varieties of banana peel has been explored by researchers. Although Ambon banana is a variety that is commonly consumed in Indonesia, the prebiotic activity of its peel, particularly the peel starch, has not been studied yet. Thus, this study aims to research the prebiotic activity of Ambon banana peel starch against probiotic bacteria of the Lactobacillus acidophilus and the opportunistic pathogen bacteria of Eschericia coli. In this research, starch was extracted from banana peel (var. pisang Ambon) and prebiotic activity of the starch was tested on L. acidophilus and E. coli. The number of bacteria was calculated at 0 and 24 hours of incubation using plate count methods.The result showed that at the concentration of 1% w/v, Ambon banana peel starch increases the number of L. acidophilus while inhibiting the growth of E. coli. The L. acidophilus culture in the starch-containing media had SCFAs (acetic, butyric and propionic acid) that were known to have good impact to human health.

Keywords: *Prebiotic; ambon banana peel; starch; L. acidophilus.*

INTRODUCTION

Prebiotics are compounds that cannot be digested but can be used by beneficial bacteria in the digestive tract so that the growth of these bacteria increases (Davani-Davari et al., 2019). The increase of beneficial bacteria in the digestive tract results in good benefits for the host's health (humans), including preventing the emergence of several diseases such as diabetes, cancer, and cardiovascular diseases (Oniszczuk et al., 2021). The magnitude of the health benefits due to the consumption of prebiotics has increased the demand for products containing prebiotics (Farias et al., 2019).

Numerous investigations have been undertaken to explore cost-effective origins of prebiotics. Agricultural wastes such as rice straw, fruit peels, and corncobs are known to be the potential source of cheap prebiotics (Le & Yang, 2019; Samanta et al., 2015). Polysaccharides or oligosaccharides contained in agricultural waste such as pectin, cellulose, starch, inulin, fructo-oligosaccharides (FOS), pectic-oligosaccharides, xylo-oligosaccharides have prebiotic effects (Bamigbade et al., 2022). Banana peel is one of the agricultural wastes which is rich in carbohydrates including starch (Aquino et al., 2016; Khamsucharit et al., 2018; Romelle et al., 2016). Bananas are one of the fruits with high production and consumption in Indonesia, thus banana peels are available in large amount (Wisnubroto, 2021).

The prebiotic effect of banana peels from several varieties have been studied. Raw “saba/kepok” banana peel powder can increase the number of Lactobacillus bacteria in vitro. A similar effect was also found in “silver bluggoe” banana peel powder (white costa banana) (Powthong et al., 2020). Banana peel of Musa sapientum (“Kluai Namwa”) variety contains resistant starch which shows a prebiotic effect against *Bacteroides vulgatus* ATCC 8482, *Bifidobacterium longum* ATCC BAA-999, *Lactobacilus casei* subsp. rhamnosus TISTR 047, and *Lactobacilus acidophilus* TISTR 450 (Jaiturong et al., 2020a) .

Ambon banana (*Musa acuminata* Triploid AAA) is a banana variety that is widely consumed in Indonesia, the fruit flesh is consumed directly or processed into other foods such as chips and cakes, but its peel is rarely used. Ambon banana contains starch, a prebiotic polysaccharide (Musita, 2012). Ambon bananas with peels increased the growth of probiotic lactic acid bacteria at concentrations of 7.5 and 10 % (Handayani, 2021). However, the prebiotic effect of starch from Ambon banana peels has not been specifically studied, thus this study aims to research the prebiotic activity of Ambon banana peel starch against probiotic bacteria of the *L. acidophilus* and opportunistic pathogen bacteria of the *E. coli*. The prebiotic effect was indicated by increasing the number of *L. acidophilus* while decreasing the number of *E. coli*. The results of this study are expected to be used as a basis for further research for the development of synbiotic products derived from Ambon banana peel waste as a supplement to treat diseases related to the immune system or metabolic disorders.

METHODS

Equipments

High performance liquid chromatography (dionex ultimate 3000 Thermo scientific), pHmeter (SevenCompactTM pH/Ion S 220, Metler-Toledo), Spectrophotometer (UV-1800, Shimadzu), oven (UNB 200, Memmert), blender (HR 2116, Phillips).

Materials

Ambon banana peels were collected from one local cake shop in Bandung, West Java. The variety of banana was determined at Sekolah Ilmu dan Teknologi Hayati (SITH), Institut Teknologi Bandung (ITB), NaOH (technical grade, Bratchem), Lactobacilus acidophilus (ATCC 4346), Escherichia coli (ATCC 25922), nutrient broth (NB, Merck), De Man, Rogosa and Sharpe (MRSA, Oxoid), nutrient agar (NA, Merck).

Methods

Starch Extraction

Starch was extracted from banana peels using the procedure described in the study conducted by Uraipan et al. (2014). Banana peels were cut into thin slices and then dried at 55 °C for 7 hours. After drying, starch extraction was carried out using NaOH (technical grade, Bratchem) and water in a ratio of 1:1. Then the slurry was filtered. The starch precipitate was rinsed several times with distilled water. Thereafter the starch was dried at 65 °C for 15 hours in an oven.

Total Carbohydrate Determination

The Phenol-sulfuric method was employed to determine the total carbohydrate concentration in the starch (Nielsen, 2017). Briefly, A100 mg/L glucose stock solution was prepared and serial dilutions were then performed by adding the glucose stock solution to distilled water. Ambon banana peel starch (40 mg) was dissolved in 10 mL of distilled water, and 1 mL of the resulting solution was mixed with 1 mL of water to create duplicates. To these solutions, 500 µL (0.05 mL) of 80 % phenol was added and vortexed. Subsequently, 5 mL of sulfuric acid was added, and the tubes were vortexed and left in a water bath at 25 °C for 10 minutes. The absorbance of each solution was measured at a wavelength of 490 nm. The absorbance of Ambon banana peel starch was substituted into the regression equation of the standard curve to quantify the total carbohydrate content.

Bacterial Rejuvenation

The prebiotic activity of starch was tested against *Lactobacilus acidophilus* (ATCC 4346), a representative of probiotic bacteria and *Escherichia coli* (ATCC 25922), as a representative of the unfavorable microflora which were obtained from Microbiology Laboratory of Pharmacy Department, Universitas Islam Bandung. De Man, Rogosa and Sharpe Broth (MRSB, Oxoid) without glucose was used as a medium for the growth of *L. acidophilus* and nutrient broth (NB, Merck) for the growth of *E. coli.* All bacteria were maintained at 37 ºC and re-cultured in fresh media every 16-24 hours.

Prebiotic Activity Test

The media containing 1 % (w/v) of Ambon banana peel starch was prepared. The media without glucose, media with 1 % (w/v) of inulin and media with 1 % (w/v) glucose were used as control. About 10 mL of each media was added with *L. acidophilus* and *E. coli* culture suspension to the final bacteria number of 1 x 10^8 CFU/mL. The cultures were incubated at 37ºC for 24 hours. The number of bacteria in the samples at 0 and 24 hours of incubation was determined using the plate count method. The media De Man, Rogosa and Sharpe agar (MRSA, Oxoid) was used for counting *L. acidophilus* and nutrient agar (NA, Merck) for counting *E. coli*. The number of bacterial colonies was calculated as the CFU/mL value. The equation 1 was used in calculating the prebiotic index (PI) of the starch (Phirom-on & Apiraksakorn, 2021).

SCFAs Analysis and pH Measurements

The SCFAs analysis was conducted at the Laboratory of Pusat Studi Pengembangan Sediaan Farmasi Fakultas Farmasi Universitas Padjadjaran. The concentration of short fatty acid (SCFA) in the form of butyrate, propionate and acetate were measured using high performance liquid chromatography under isocratic conditions using C_{18} column (Universal Fortis) and measured at a Column C_{18} 15 cm, wave length 210 nm, mobile phase 5 % acetonitrile in 0.012 N H_2SO_4, flow rate 0.5 mL/min., and injection volume 20 µL. The pH value of bacterial culture solution containing banana peel starch was measured using a pH meter at 0, 24, 48 and 72 hours of incubation.

Statistical Analysis

The difference of bacteria number between groups were analyzed statistically using ANOVA followed with Tukey Test. The difference of *L. acidophilus* and *E. coli* number in media with Ambon banana peel starch was analyzed using T-Test.

$$\mathrm{PI} = \left[\frac{(P_p^{24}-P_p^0)-(P_{GF}^{24}-P_{GF}^0)}{(P_G^{24}-P_G^0)-(P_{GF}^{24}-P_{GF}^0)}\right] - \left[\frac{(E_p^{24}-E_p^0)-(E_{GF}^{24}-E_{GF}^0)}{(E_G^{24}-E_G^0)-(E_{GF}^{24}-E_{GF}^0)}\right] \quad (1)$$

Where:

P_p^0, P_p^{24} = CFU for probiotic (*L. acidophilus*) at 0 and 24 h in media enriched with starch
P_{GF}^0, P_{GF}^{24} = CFU for probiotic (*L. acidophilus*) at 0 and 24 h in glucose-free media
P_G^0, P_G^{24} = CFU for probiotic (*L. acidophilus*) at 0 and 24 h in media enriched with glucose
E_p^0, E_p^{24} = CFU for pathogen (*E. coli*) at 0 and 24 h in media enriched with starch
E_{GF}^0, E_{GF}^{24} = CFU for pathogen (*E. coli*) at 0 and 24 h in glucose-free media
E_G^0, E_G^{24} = CFU for pathogen (*E. coli*) at 0 and 24 h in media enriched with glucose

RESULTS AND DISCUSSION

The determination of the banana variety being used in this research by Sekolah Ilmu dan Teknologi Hayati (SITH), Institut Teknologi Bandung (ITB) showed that it was *Musa acuminata* (AAA Group) 'Ambon' (number of certificate 5214/IT1.C11.2/TA.00/2022). The yield of starch from fresh Ambon banana peel was 0.67 %. While the total carbohydrate content in the starch was 44.26 % w/w. The effect of starch Ambon banana peel as a carbon source was tested on the growth of the probiotic bacteria, *L. acidhopilus* and the opportunist microbiota *E. coli*. In this test, glucose is employed as the positive control, as it is known to be a carbon source that can be utilized by various types of bacteria (Sánchez-Clemente et al., 2020). Inulin is used as a reference because it is a known prebiotic compound that can enhance the growth of beneficial bacteria (Pandey et al., 2015). Bacteria grown on media without glucose are used as the negative control. It is hypothesized that bacteria cultivated in this medium will experience slower growth compared to bacteria grown in media containing a carbon source.The results are presented in Table 1.

ANOVA analysis results showed that the number of *L. Acidophilus* that grew in media containing Ambon banana peel starch were significantly higher than the bacteria in media with inulin or without carbon source. The number of *L. Acidophilus* in media with Ambon banana peel starch was not significantly different to glucose. Meanwhile the *E. coli* number in media with Ambon banana peel starch was significantly lower than in media media without carbon source and/or inulin. Furthermore, the statistical analysis (T-test) of bacterial count in cultures of *L. acidophilus* and *E. coli* treated with Ambon banana peel starch, indicates that the quantity of *L. acidophilus* bacteria was significantly higher than that of *E. coli*. Thus, it can be concluded that Ambon banana peel starch has prebiotic activity because it can increase the growth of beneficial bacteria (*L. acidophilus*) and suppress the growth of opportunistic bacteria (*E. coli*). While, the PI of Ambon banana peel starch was 0.21. This result supports findings in the previous study which reported that banana peels had a prebiotic effect on lactic acid bacteria (LAB (Zahid et al., 2021). However, the PI of Ambon banana peel starch was lower than reported by previous research which used unripe banana of Kluai Namwa Luang (pisang awak) variety (Jaiturong et al., 2020b). This might be due to the starch used in this study was from ripe bananas while the ripe banana was known to have lower content of prebiotic in the form of resistant starch than unripe banana (Li et al., 2020; Phillips et al., 2021).

Table 1. Effect of Ambon Banana Peel Starch on The Growth of *L. acidophilus* and *E. coli* After 24 Hours of Incubation

Bacteria	Number of Bacteria (Log CFU/mL)				
	0 h (for all groups)	24 h			
		Without carbon source	Glucose	Inulin	Ambon banana peel starch
L. acidophilus	8 ± 0	6.62 ± 0.24	8.13 ± 0.26*	7 ± 0.52	9.11 ± 0.71*
E. coli	8 ± 0	11.86 ± 0.78	8.34 ± 0.48*	10.57 ± 0.10	6.81 ± 0.22*

Data are in mean ± SD (n=3). *) is significantly different compared to bacteria in media without carbon source/inulin ($p < 0.5$)

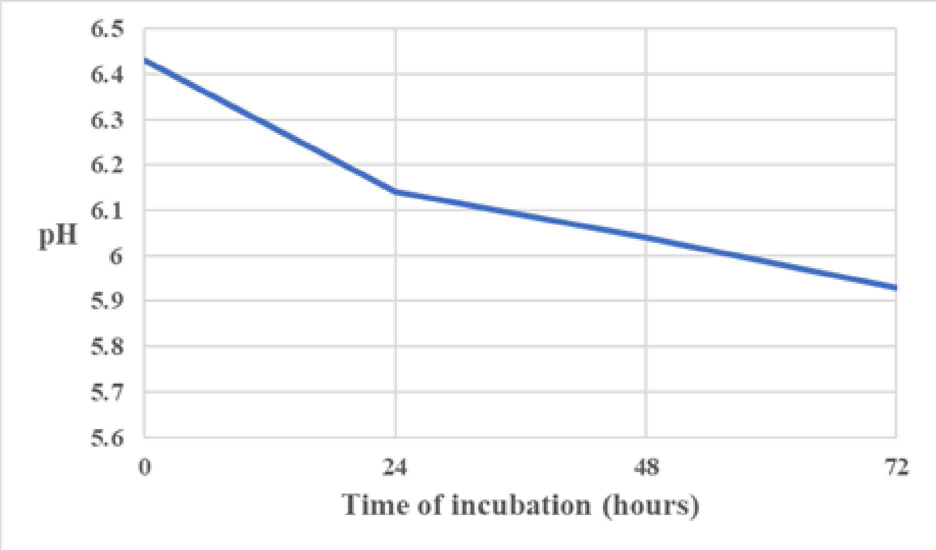

Figure 1. The pH of *L. acidophilus* culture that enriched with Ambon banana peel starch

Inulin was reported as a potential prebiotic and could selectively increase *L. acidophilus* (Carlson et al., 2018; Setiarto et al., 2017; Zarinah et al., 2018). However, in this study, inulin showed no prebiotic effect, and its PI was – 0.11. The negative result of inulin prebiotic activity was also reported in previous studies (Adebola et al., 2014; Bedani et al., 2013).

Lactobacilli converts polysaccharides into SCFA (Zhao et al., 2021). Increased levels of SCFA might be indicated by a decrease in the pH of the bacterial culture solution. In 72 hours, pH of *L. acidophilus* culture that contains Ambon banana peel starch was declining (Figure 1.). Furthermore, the concentration of SCFA in the culture after 24 hours incubation was determined (Table 2).

Table 2. Concentration of SCFA in *L. acidophilus* culture that enriched with Ambon banana peel starch

SCFA	Concentration (µg/mL)		
	1	2	mean
Acetic acid	8.03	10.63	9.33
Butyric acid	5.69	6.06	5.88
Propionic acid	0.81	0.77	0.79

Acetic acid was the highest SCFA contained in the *L. acidophilus* culture media enriched with 1 % b/v Ambon banana peel starch followed by butyric acid and lastly propionic acid. This trend was similar to SCFAs content of human faecal slurry incubated with banana flesh powder (*Musa acuminata Colla*) 0.2 % b/v (Tian et al., 2020). Acetic acid is a SCFA known to play a role in controlling body weight and insulin sensitivity (Hernández et al., 2019). Butyric acid, which is a source of energy for intestinal cells, can improve intestinal peristalsis and has anti-inflammatory activity (Pituch et al., 2013; Xu et al., 2021; Załęski et al., 2013). While propionic acid is associated with lowering blood lipid levels and preventing cancer (Hosseini et al., 2011). This finding suggests that Ambon banana peel starch might exert a beneficial effect on health.

CONCLUSIONS

Ambon banana peel starch had a prebiotic effect in vitro which can be seen from their ability to increase the number of *L. acidophilus* and suppress the growth of *E. coli*. Acetic acid, butyric acid and propionic were detected in the *L. acidophilus* culture which grew in Ambon banana peel starch containing media. The presence of those SCFAs were also indicated by pH reduction over 72 hours period of incubation. Further study in the animal model is needed to confirm the prebiotic effect of Ambon banana peel starch.

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