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Prebiotic Activity of Ambon Lumut Banana (*Musa acuminata* AAA) Peel Pectin

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Abstract

Banana peel is an agricultural waste that contains pectin. Ambon lumut banana (*Musa acuminata* AAA) is often consumed in Indonesia, but its peel is rarely utilised; thus, the availability of Ambon lumut banana peel in Indonesia is high. Pectin selectively increases beneficial gut bacteria, commonly known as a prebiotic. This study aims to evaluate the prebiotic activity of Ambon lumut banana peel by observing the growth of a healthy gut bacteria, *Lactobacillus acidophilus*, and enteric pathogen bacteria, *Escherichia coli*, in media (MRSB) enriched with the pectin. The result showed that 1% of Ambon lumut banana peel pectin significantly increased the number of *L. acidophilus* and decreased the number of *E. coli* compared to bacteria culture without carbon source (glucose-free MRSB) and bacteria culture with glucose as carbon source. The prebiotic index of Ambon lumut banana peel pectin was 0.53. In addition, the short-chain fatty acid (SCFA), which is a metabolite of *L. acidophilus* for human health, was also measured using HPLC. The HPLC analysis showed that *L. acidophilus* culture enriched with pectin contains SCFA, acetic acid, butyric acid, and propionic acid at 10.22 µg/mL, 5.38 µg/mL, and 0.55 µg/mL, respectively. This study shows that pectin of Ambon lumut banana peel had prebiotic activity indicated by its ability to increase the growth of beneficial bacteria significantly. Thus, this is a good chance to develop new sources for prebiotic formulation.

Keywords: Pectin, Ambon lumut banana peel, Prebiotic, *Lactobacillus acidophilus*.

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Introduction

Prebiotics are undigestible substances that selectively increase the growth of beneficial bacteria in the gut.[1] Carbohydrates are the most common prebiotic substances. Pectin, cellulose, inulin, fructo oligosaccharides (FOS), pectic-oligosaccharides, and xylooligosaccharides are well-known prebiotic polysaccharides.[2] These substances are fermented into SCFA by beneficial gut bacteria. SCFA is involved in the metabolism of humans, resulting in a positive impact on human health.[3] Thus, increased beneficial gut bacteria can prevent diabetes, cancer, and cardiovascular diseases.[4] Due to the good effect of prebiotics, the demand for a product containing prebiotics is rising.[5]

Many researchers have discovered cheap prebiotic sources. Agricultural waste like rice straw, fruit peel, and corn cobs was found to have high prebiotic carbohydrates. Pectin is a high-molecular-weight carbohydrate polymer in virtually all plants where it contributes to the cell structure. Pectin is mainly found in fruits and is particularly rich in its peel. Banana peel yielded pectin up to 24% of its dried powder.[6] Hence this agricultural waste is a promising alternative pectin source. Furthermore, the prebiotic activity of banana peel pectin in vitro has been reported by a former study.[7] However, the variant of banana used in that study was not mentioned.

Pisang Ambon lumut (Ambon lumut banana/ *Musa acuminata* AAA) is often consumed in Indonesia. However, utilisation of its peel is still limited and mostly wasted. A previous study revealed that Ambon lumut banana peel contains 14.9% of pectin (8), but the prebiotic activity of Ambon lumut banana peel pectin has not been studied. Thus, this study aims to investigate the prebiotic activity of pectin Ambon lumut banana peel against *Lactobacillus acidophilus* (*L. acidophilus*) and *Escherichia coli* (*E. coli*) and measure the level of Short Chain Fatty Acid (SCFA) in the *L. acidophilus* culture media.

Methods

Pectin Extraction

Ambon lumut banana peels were collected from a banana cake factory in Bandung, West Java. The variety of the banana was confirmed by the School of Life Sciences and Technology of Bandung Institute of Technology (SITH-ITB), as *Musa acuminata* (AAA Group) 'Ambon'. Pectin was extracted from Ambon lumut banana peel using the method developed by Oliviera et al.[9] A total of 1500 g of ground banana peel were washed three times with ethanol (Bratachem, technical grade) to remove alcohol-soluble phenolic compounds. Then they were washed with acetone (Bratachem, technical grade).

Furthermore, the residue (dregs) that had been washed was dried at room temperature. Pectin was extracted from the dry residue using a solution of citric acid (Bratachem, technical grade) to the ratio of 1:20 in a water bath at 83°C with stirring for 190 minutes. The supernatant was separated by centrifugation, and absolute ethanol (Sigma Aldrich, pro-Analisa) was added to the supernatant and stirred until the pectin dissolved. The pellets were collected and washed with 70% (v/v) ethanol (Bratachem, technical grade) and dried at room temperature. After drying, they were dispersed in water, and the pH of the mixture was adjusted to 7 (with KOH), then dried again and ground to a powder form.

Prebiotic Activity Assay

L. acidophilus (ATCC 4356) represents beneficial bacteria (probiotics). *E. coli* (ATCC 25922) was used to represent the unfavorable microflora. De Mann Rogosa and Sharpe Broth (MRSB, Oxoid) without glucose were used as a medium for the growth of *L.*

acidophilus and Nutrient Broth (NB, Merck) for the growth of *E. coli*. Media with glucose was used as a control. About 9 mL of MRSB was added with 1 mL culture of L. acidophilus. The final number of L. acidophilus was 10^8 CFU/mL. Pectin was added to the culture at a concentration of 1% W/V. A similar number of bacteria and concentration of pectin was used for the *E. coli* culture. Both cultures were incubated at 37°C for 24 hours. Samples were taken at the end of the incubation period to calculate the pH, SCFA content analysis, and bacterial number. The bacterial number was counted using the drop plate method. De Mann Rogosa and shape Agar (MRSA, Oxoid) was used for *L. acidophilus* counting, and Nutrient Agar (NA, Merck) was used for E. coli counts. Plates were incubated at 37°C for 24 hours. The number of bacterial colonies was calculated as the CFU/mL value. The pH was measured using pH indicator paper. The prebiotic activity score (PI) of prebiotics (pectin and inulin) was calculated using the following equation[10,11]

Short Chain Fatty Acid (SCFA) Analysis

$$PI = \left[\frac{(P_P^{24}-P_P^0)-(P_{GF}^{24}-P_{GF}^0)}{(P_G^{24}-P_G^0)-(P_{GF}^{24}-P_{GF}^0)}\right] - \left[\frac{(E_P^{24}-E_P^0)-(E_{GF}^{24}-E_{GF}^0)}{(E_G^{24}-E_G^0)-(E_{GF}^{24}-E_{GF}^0)}\right]$$

Where:

P_p^0, P_p^{24} = CFU for probiotic (*L. acidophilus*) at 0 and 24 h in prebiotic containing media

P_{GF}^0, P_{GF}^{24} = CFU for probiotic (*L. acidophilus*) at 0 and 24 h in glucose-free containing media

P_G^0, P_G^{24} = CFU for probiotic (*L. acidophilus*) at 0 and 24 h in glucose containing media

E_p^0, E_p^{24} = CFU for pathogen (*E. coli*) at 0 and 24 h in prebiotic containing media

E_{GF}^0, E_{GF}^{24} = CFU for pathogen (*E. coli*) at 0 and 24 h in glucose-free containing media

E_G^0, E_G^{24} = CFU for pathogen (*E. coli*) at 0 and 24 h in glucose containing media

The SCFAs were analysed according to a procedure previously described by Guerrant et al.[12] with modification. The SCFAs were extracted from *L. acidophilus* culture as follows: 2 mL of broth culture was added with 0.2 mL of 18 N H2SO4 (Merck, pro Analisa), 0.6 g of NaCl (Merck, pro Analisa), 5 mL of diethyl ether (Merck, pro Analisa), and 25 µL of acetonitrile (Merck, pro HPLC); the mixture was homogenised with a Vortex mixer for 1 minute. The ether phase was collected and added with 0.2 mL of 0.1 N NaOH (Merck, pro Analisa). The mixture was blended and centrifuged, the pH was checked, and if necessary 1 N NaOH was added until the pH was 9 or greater. The ether phase was removed and added with 25 µL of acetonitrile. The tube was left uncapped to allow the residual ether to evaporate. The mixture was then blended with a vortex mixer, and 20 µL of the mixture was introduced to high-performance liquid chromatography (HPLC, dionex ultimate 3000 Thermo Scientific) using a C18 column (Universal Fortis). The mobile phase was acetonitrile 5% in 0.012 N H2SO4, with a flow rate of 0.5 mL/minute. Acetic acid, butyric acid, and propionic acid were detected using a UV-Vis detector at a wavelength of 210 nm. The concentration of the SCFAs was determined by inserting the Area Under Curve (AUC) obtained from samples using the linear equation of related SCFA standards.

Statistical Analysis

All measurements of each parameter were performed in triplicate. Data were presented in the form of mean with deviation (SD). Data were analysed statistically using analysis of variance (ANOVA) and Tukey's test to analyse significant differences in prebiotic activity (growth of *L. acidophilus* and *E. coli* after treatment). Differences between the growth of *L. acidophilus* and *E. coli* in each treatment were analysed with a t-test.

Results and Discussion

Prebiotics are a group of nutrients that are degraded by gut microbiota. Their relationship with overall human health has been an area of increasing interest in recent years. They can feed the intestinal microbiota, and their degradation products are short-chain fatty acids released into blood circulation, consequently affecting the gastrointestinal

tracts and other distant organs. Fructo-oligosaccharides, galactooligosaccharides, and pectin are the two important prebiotics with beneficial effects on human health.[13] In this study, we extracted Ambon lumut banana peel to investigate its prebiotic activity.

Ambon lumut banana yielded 0.2% of pectin from the fresh peel (mass of pectin/mass of fresh peel). The yield was lower than previously reported by Tuhuloula *et al*.[14], probably due to different extraction methods and sources of bananas. The present study used citric acid as a solvent, while Tuhuloula *et al*.[14] used chloric acid. The heating time was also different, Tuhuloula *et al*.[14] heated the extract for 2 hours, while the extraction time in the current study was 1.5 hours. Extraction yield might vary depending on parameters such as pH, temperature, heating time, sample provenance, and liquid/solid ratio.[14] There are wide varieties of Ambon banana; the present study used Ambon lumut banana, while the previous study did not mention the variety. Different origins, variety, and environmental growth conditions of bananas might cause another pectin yield.[15] A survey of optimisation of the parameter of pectin extraction from Banana Ambon lumut peel is needed to increase the pectin yield.

The results of the prebiotic activity assay are shown in Table 1. Pectin of Ambon lumut banana had a similar number of *L. acidophilus* to glucose, but significantly higher than inulin and glucose free. The highest *L. acidophilus* growth was observed in 1% pectin. This finding aligns with a previous study, which reported that the 0.1% w/v hydrolysed pectinase of banana peel showed a growth-enhancing effect on lactic acid bacteria (*L. paracasei*, *L. rhamnosus*, and *L. lactis*).[7]

The growth of *E. coli* in media containing pectin was significantly lower than in glucose-free media and inulin. Pectin of Ambon lumut banana peel significantly had a higher number of *L. acidophilus* than *E. coli*. Media MRS without a carbon source (glucose-free) had the lowest number of *L. acidophilus* and the highest number of *E. coli* compared to media with a carbon source (glucose, inulin, or pectin). These results confirmed that Pectin from Ambon lumut banana peel has prebiotic activity *in-vitro* because it can selectively increase the number of *L. acidophilus* and lower the number of *E. coli* with a prebiotic index of 0.53.

Inulin is a well-known known prebiotic.[16] Previously, this substance increased the number of *L. acidophilus*[17] and decreased the number of *E. coli*[18], however, in the present study, the results were contrary. No significant effect of inulin on the growth of *L. acidophilus*. It might be because the pectin used was taken from supplements, thus, the puroty can not be guaranteed. Further studies are necessary to clarify these results because there were similar results reported priorly.[19,20]

Table 1. Prebiotic Assay Result of Ambon Lumut Banana Pectin

Bacteria	Number of Bacteria (log CFU/mL) after Incubated with			
	No Glucose	1% Glucose	1% Inulin	1% Pectin of Ambon Lumut Banana Peel
L. acidophilus	6.62 ± 0.24	8.13 ± 0.27*	7 ± 0.52	9.44 ± 0.67*
E. coli	12.26 ± 0.78*	7.3 ± 0.48	10.57 ± 0.1*	7.15 ± 0.05

Data presented in a mean of triplicate measurements ± SD; *) significantly different (p<0.05)

The pH of the pectin-containing culture trended to slightly decline during 72 hours of incubation (Figure 1). The decline might be related to the production of SCFAs by *L. acidophilus*. The HPLC analysis revealed that 24 H old *L. acidophilus* culture in pectin-enriched media contained SCFAs, the acetic acid, butyric acid, and propionic acid. The concentration of acetic acid, butyric acid, and propionic acid were 10.22 µg/mL, 5.38 µg/mL, and 0.55 µg/mL, respectively. The genera *Lactobacillus* produces these SCFAs via glycolysis of the prebiotic carbohydrates.[22] Acetic, butyric, and propionic acid play important roles in human health.[23] Acetic acid is controlling body weight and insulin sensitivity.[24] Butyric acid is a primary energy source for colonocytes.[25] This substance improves bowel movement and has a potent antiinflammation effect. Thus, butyrate supplements might help with digestive disorder therapy.[25,26,27] Propionic acid is suggested to decrease lipogenesis, serum cholesterol level, and carcinogenesis beyond the gut tissues.[28]

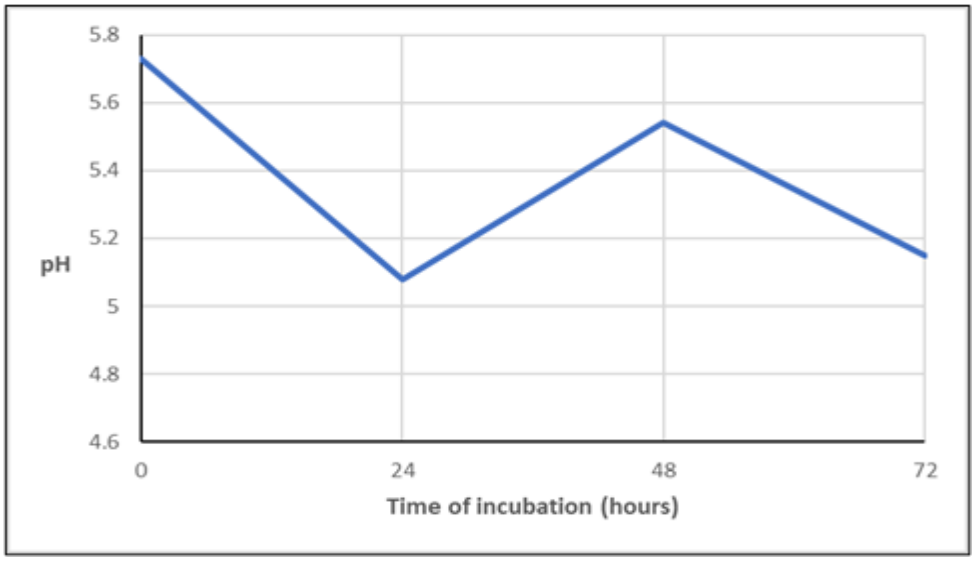

Figure 1. pH of *L. acidophilus* culture in the Ambon lumut banana peel pectin-containing media

Conclusion

Pectin of Ambon lumut banana peel had prebiotic activity indicated by its ability to significantly increases the growth of beneficial bacteria, *L. acidophilus*, and suppress the growth of *E. coli* with the prebiotic index of 0.53 of the SCFAs, including acetic acid, butyric acid, and propionic acid, was detected in the media of *L. acidophilus* culture containing pectin from Ambon lumut banana peel. The concentration of acetic acid was 10.22 µg/mL, butyric acid was 5.38 µg/mL, and propionic acid was 0.55 µg/mL.

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Conflict of Interest
None declared.

References

1. Roberfroid M. Prebiotics: The Concept Revisited. *The Journal of Nutrition*. 2007; 137(3): 830S-837S. DOI: 10.1093/jn/137.3.830S
2. Wichienchot S, Wan Ishak WR. Prebiotics and Dietary Fibers from Food Processing By-Products. In: ed. Anal AK. Food Processing By□Products and their Utilization. 1st ed. Hoboken: Wiley-Blackwell; 2017. p. 137–74.
3. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell*. 2016; 165(6): 1332–45. DOI: 10.1016/j.cell.2016.05.041
4. Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, *et al*. Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods*. 2019; 8(3): 92. DOI: 10.3390%2Ffoods8030092
5. Azmi AFMN, Mustafa S, Hashim DM, Manap YA. Prebiotic activity of polysaccharides extracted from Gigantochloa levis (Buluh beting) shoots. Molecules. 2012;17(2):1635–51.
6. Khamsucharit P, Laohaphatanalert K, Gavinlertvatana P, Sriroth K, Sangseethong K. Characterization of pectin extracted from banana peels of different varieties. *Food Science and Biotechnology*. 2018; 27(3): 623–9. DOI: 10.1007%2Fs10068-017-0302-0
7. Doan CT, Chen CL, Nguyen VB, Tran TN, Nguyen AD, Wang SL. Conversion of Pectin-Containing By-Products to Pectinases by Bacillus amyloliquefaciens and Its Applications on Hydrolyzing Banana Peels for Prebiotics Production. *Polymers*. 2021; 13(9): 1483. DOI: 10.3390/polym13091483
8. Tuhuloula A, Budiyarti L, Fitriana EN. Karakterisasi pektin dengan memanfaatkan limbah kulit pisang menggunakan metode ekstraksi. *Konversi*. 2013; 2(1): 21–7. DOI: 10.20527/k.v2i1.123
9. Lopes SMS, Francisco MG, Higashi B, de Almeida RTR, Krausová G, Pilau EJ, *et al*. Chemical characterization and prebiotic activity of fructo-oligosaccharides from *Stevia rebaudiana* (Bertoni) roots and *in vitro* adventitious root cultures. *Carbohydrate Polymers*. 2016; 152: 718–25. DOI: 10.1016/j.carbpol.2016.07.043
10. Huebner J, Wehling RL, Parkhurst A, Hutkins RW. Effect of processing conditions on the prebiotic activity of commercial prebiotics. *International Dairy Journal*. 2008; 18(3): 287–93. DOI: 10.1016/j.idairyj.2007.08.013
11. Phirom-on K, Apiraksakorn J. Development of cellulose-based prebiotic fiber from banana peel by enzymatic hydrolysis. *Food Bioscience*. 2021; 41: 101083. DOI: 10.1016/j.fbio.2021.101083
12. Guerrant GO, Lambert MA, Moss CW. Analysis of short-chain acids from anaerobic bacteria by high-performance liquid chromatography. *Journal of Clinical Microbiology*. 1982; 16(2): 355–60. DOI: 10.1128%2Fjcm.16.2.355-360.1982
13. Slavin J. Fiber and Prebiotics: Mechanisms and Health Benefits. *Nutrients*. 2013; 5(4): 1417-35. DOI: 10.3390%2Fnu5041417
14. Robledo VR, Vázquez LIC. Pectin - Extraction, Purification, Characterization and Applications. In: ed. Masuelli MA, Pectins - Extraction, Purification, Characterization and Applications. Rijeka: IntechOpen; 2019. ch. 3.
15. Chan SY, Choo WS. Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. *Food Chemistry*. 2013; 141(4): 3752–8. DOI: 10.1016/j.foodchem.2013.06.097

16. Carlson JL, Erickson JM, Lloyd BB, Slavin JL. Health Effects and Sources of Prebiotic Dietary Fiber. *Current Developments in Nutrition*. 2018; 2(3): nzy005. DOI: 10.1093/cdn/nzy005
17. Setiarto RHB, Widhyastuti N, Saskiawan I, Safitri RM. Pengaruh Variasi Konsentrasi Inulin Pada Proses Fermentasi Oleh *L. Acidophilus*, *L. Bulgaricus* Dan *S. Thermophillus*. *Biopropal Industri*. 2017; 8(1): 1–17.
18. Zarinah Z, Anis AA, Napisah H, Shazila S. Prebiotic activity score of breadfruit resistant starch (*Artocarpus altilis*), breadfruit flour, and inulin during *in-vitro* fermentation by pure cultures (*Lactobacillus plantarum*, and *Bifidobacterium bifidum*). *Journal of Agrobiotechnology*. 2018; 9(1S): 122–31.
19. Adebola OO, Corcoran O, Morgan WA. Synbiotics: the impact of potential prebiotics inulin, lactulose and lactobionic acid on the survival and growth of lactobacilli probiotics. *Journal of Functional Foods*. 2014; 10: 75–84. DOI: 10.1016/j.jff.2014.05.010
20. Bedani R, Rossi EA, Saad SMI. Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions. *Food Microbiology*. 2013; 34(2): 382–9. DOI: 10.1016/j.fm.2013.01.012
21. Blanco-Pérez F, Steigerwald H, Schülke S, Vieths S, Toda M, Scheurer S. The Dietary Fiber Pectin: Health Benefits and Potential for the Treatment of Allergies by Modulation of Gut Microbiota. *Current Allergy and Asthma Reports*. 2021; 21(10): 43. DOI: 10.1007/s11882-021-01020-z
22. Wang L, Cen S, Wang G, Lee YK, Zhao J, Zhang H, *et al*. Acetic acid and butyric acid released in large intestine play different roles in the alleviation of constipation. *Journal of Functional Foods*. 2020; 69: 103953. DOI: 10.1016/j.jff.2020.103953
23. Zhu L, Baker RD, Baker SS. Gut microbiome and nonalcoholic fatty liver diseases. *Pediatric Research*. 2015; 77(1): 245–51. DOI: 10.1038/pr.2014.157
24. Hernández MAG, Canfora EE, Jocken JWE, Blaak EE. The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients*. 2019; 11(8): 1943. DOI: 10.3390/nu11081943
25. Pituch A, Walkowiak J, Banaszkiewicz A. Butyric acid in functional constipation. *Przeglad Gastroenterologiczny*. 2013; 8(5): 295–8. DOI: 10.5114%2Fpg.2013.38731
26. Xu HM, Huang HL, Xu J, He J, Zhao C, Peng Y, *et al*. Cross-Talk Between Butyric Acid and Gut Microbiota in Ulcerative Colitis Following Fecal Microbiota Transplantation. *Frontiers in Microbiology*. 2021; 12: 658292. DOI: 10.3389/fmicb.2021.658292
27. Załęski A, Banaszkiewicz A, Walkowiak J. Butyric acid in irritable bowel syndrome. *Przeglad Gastroenterologiczny*. 2013; 8(6): 350–3. DOI: 10.5114%2Fpg.2013.39917
28. Hosseini E, Grootaert C, Verstraete W, Van de Wiele T. Propionate as a health-promoting microbial metabolite in the human gut. *Nutrition Reviews*. 2011; 69(5): 245–58. DOI: 10.1111/j.1753-4887.2011.00388.x