Pharmacology and Clinical Pharmacy Research
Volume 8 No. 2 August 2023
ISSN:2527-7322 | e-ISSN: 2614-0020
doi: 10.15416/pcpr.v8i3.49261

The Effect of Prebiotic Starch and Pectin from Ambon Banana Peel (*Musa acuminata AAA*) on The Growth of Skin Microbiota Bacteria In Vitro

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Abstract

Propionibacterium acnes is a bacteria which causes acne. This bacteria is hypothesized to be inhibited by *Staphylococcus epidermidis (S. epidermidis)*. Prebiotics have been shown to enhance the number of *S. epidermidis* and decrease the growth of *Propionibacterium acnes (P. acnes)*. The prebiotic action of starch and pectin from diverse plant sources is known in the skin microbiome. The prebiotic activity of Ambon banana peel starch and pectin on skin microbiota has not been researched. This study aims to investigate the prebiotic activity of starch and pectin from Ambon banana peels on skin microbiota, represented by *S. epidermidis* and *P. acnes*. The results showed that starch, and pectin have a prebiotic activity because they promoted the growth of *S. epidermidis* while suppressing the growth of *P. acnes*. *P. acnes* inhibitor percentages were 1.62% for starch and 65.07% for pectin. Negative inhibition values were -184.95% for starch and -5.80% for pectin suggesting an increase in S. *epidermidis* proliferation.

Keywords: ambon banana; starch; pectin; prebiotic

Introduction

Acne is a common skin disorder that occurs during adolescence. Various factors can trigger the development of acne, including genetics, hormones, diet, and environmental factors (Dipiro, 2020). Four stages characterize the process of acne formation: increased sebum production, colonization by *P. acnes*, inflammation formation due to the release of inflammatory mediators, and follicular keratinization process.[1]

P. acnes produces lipase that hydrolyzes sebum triglycerides into fatty acids. These fatty acids trigger keratinization and the formation of microcomedones (damage to sebaceous glands and follicles). Closed comedones are the first visible acne lesions. *P. acnes* triggers inflammation due to immune response.[1] Recent studies have shown that *S. epidermidis* play a role in the pathophysiology of acne. Acne can be caused by an imbalance of the skin microbiome between *S. epidermidis* and *P. acnes*. Both *S. epidermidis* and *P. acnes* produce short-chain fatty acids (SCFAs) that act as antimicrobials against each other. These two bacteria are found on acne-prone skin. There is no evidence that *S. epidermidis* is the

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Received: 13 August 2023 **Revised**: 25 October 2023 **Published:** 30 November 2023

ISSN:2527-7322 | e-ISSN: 2614-0020

cause of acne formation.[2] Wang et al. (2016) states that *S. epidermidis* can ferment glycerol to produce SCFAs that suppress the growth of *P. Acnes*.[3]

Current acne treatments often involve the use of oral and topical antibiotics. However, recent developments have shown increased resistance to macrolide antibiotics such as clindamycin, used for acne treatment. Approximately 50% of *P. acnes* strains have developed resistance, decreasing antibiotic effectiveness.[4] The use of probiotics in acne treatment has shown positive results by controlling the growth of them. The oral use of probiotics as an adjuvant therapy to existing treatments can improve the healing of mild to moderate acne. Probiotics can produce bacteriocin substances, which are antibacterial proteins that inhibit the growth of *P. acnes*. Probiotics also inhibit IL-8 and TNF-alpha cytokines in epithelial cells and keratinocytes, reducing inflammatory reactions.[4]

Prebiotics are substances that can enhance the growth of probiotics. The use of prebiotics in acne treatment shows promising potential due to their ability to increase the growth of probiotics. Banana peel is one prebiotic studied for its role in acne therapy. Ethanol extract of yellow banana peel (*Musa balbisiana*) has been found to inhibit the growth of *P. acnes* at a concentration of 10%.[5] Ethanol extract of Ambon banana peel at concentrations starting from 10% inhibits the growth of *P. acnes*.[6] Traditional use of banana peel by rubbing the inner part of the peel on the acne-prone face in 45 teenage girls can reduce the severity of acne after seven days of application.[7]

Research on the benefits of banana peel prebiotics against bacteria involved in acne formation is still limited. This study demonstrates the benefits of starch and pectin in Ambon banana peel in restoring the balance of *P. acnes* and *S. epidermidis* growth.

Methods

Banana peels were obtained from a banana cake bakery in Bandung, West Java. The banana species from these peels is Musa acuminata (AAA group) 'Ambon' based on the determination by the School of Life Sciences and Technology (SITH), Bandung Institute of Technology (ITB), certificate number: 5214/IT1.C11.2/TA.00/2022.

Pectin Preparation

Pectin was extracted using a procedure developed by Saches Lopes et al. (2016).[8] The banana peels were ground after thorough washing and then washed three times with ethanol (Bratachem, technical grade). At each washing step, the material was filtered using cheesecloth. The washed residue was subsequently dried at room temperature. After drying, the pectin was extracted using a citric acid solution (pH 2.7) (Bratachem, technical grade) with a 1:20 w/v ratio for 190 minutes at 83°C with stirring.

The solution was then centrifuged (1147 g, 30 minutes, 10°C) to separate the supernatant. The supernatant was vacuum-filtered, absolute ethanol (Sigma Aldrich, pro analysis) was added, and the pH was adjusted to 3.5 using KOH (Merck, pro analysis). The mixture was stirred for 30 minutes, allowed to settle for 2 hours at 4°C, and then centrifuged for 15 minutes at 3500 rpm. The resulting pellet was collected, washed with 70% ethanol (Bratachem, technical grade), and centrifuged again (20 minutes, 3500 rpm). The pellet was dried at room temperature. Once dry, the pellet was dispersed in pH 7 water (adjusted with KOH) and dried again.

Starch Preparation

Starch was prepared following the procedure conducted by Uraipan et al. (2014).[9] The banana peels were sliced into small pieces and then dried at 55°C for 7 hours. After drying,

the starch was extracted using a 1:1 ratio of NaOH (Bratchem, technical grade) and water. The slurry was then filtered, and the starch precipitate was rinsed several times with distilled water. The starch was subsequently dried in an oven at 65°C for 15 hours.

Bacterial Preparation

The bacteria used in this study were *S. epidermidis* (ATCC12228) and *P. acnes* (ATCC11827) obtained from the Microbiology Laboratory, Islamic University of Bandung. Both bacteria were cultured in Tryptic Soy Broth (TSB, Oxoid) and incubated overnight at 37°C under aerobic conditions. After incubation, the media from each culture were collected and diluted with their respective media at a dilution ratio 1:10. The diluted samples were ready for antibacterial testing. The pH of *S. epidermidis* cultures were measured at 0, 3, and 24 hours.

Antibacterial Testing

The antibacterial testing method followed the procedure reported by Di Lodovico et al. (2020).[10] The absorbance of the diluted cultures of *S. epidermidis* and *P. acnes* was measured using a UV spectrophotometer (Shimadzu UV-1800, Japan) at a wavelength of 600 nm (optical density, OD600), adjusted to OD600 = 0.12. Then, a solution of media containing 2% w/v starch/pectin was added to the bacterial suspension at a 1:1 ratio, resulting in a final concentration of 1% w/v starch/pectin in the culture solution.

Cultures without adding starch/pectin were used as controls, and TSB media served as blanks. The OD600 of the samples and controls was measured at 0 hours, and then the cultures were incubated at 37°C for 24 hours. OD600 measurements were also taken at 3 and 24 hours. Each treatment and measurement was performed in duplicate.

The percentage of inhibition was calculated using the following formula:

Percentage of inhibition (%) = [(ODcontrol - ODsample) / ODcontrol] × 100. The OD600 value and the percentage of inhibition were statistically analyzed using Kruskall-Walis because the data were not normally distributed.

Results and Discussion

The Prebiotic Effect of Ambon Banana Starch and Pectin

The ability of starch and pectin in Ambon banana peel to selectively enhance the number of beneficial bacteria is called prebiotic effect. In this research, in vitro studies were performed on *P. acnes*, a skin microbiota that causes acne[11] and *S. epidermidis*, which represent beneficial microflora bacteria.[12] This is about earlier study findings showing *S. epidermidis* can prevent the skin against acne.[11] This study selected glucose as a standard carbon source required for bacterial growth[13] and inulin as an established and frequently utilized prebiotic.[14]

The OD600 was used to monitor the development of the test bacteria. The number of bacteria is related to the OD600 value. The OD600 value increases with the number of bacteria. Figure 1 shows starch had an average OD600 value lower than control, glucose, and inulin after 3 hours of incubation. However, after 24 hours of incubation, the OD600 value of starch was not significantly different from inulin and control but remained lower than glucose.

At the 3rd and 24th hours of incubation, the average OD600 value of the *P. acnes* culture treated with Ambon banana peel pectin was lower than the control group or inulin as a standard prebiotic but not statistically significant ($p>0.05$) compared to another group. Based on the OD600 value, the least number of *P.acnes* bacteria were observed in the cultures treated with pectin for 24 hours of incubation.

At the same incubation hour, the number of *P. acnes* exposed to starch was equivalent to that of *P.acnes* exposed to inulin and the control (without additional carbon sources), but fewer than that of *P. acnes* exposed to glucose.

Figure 2 depicts the influence of Ambon banana peel starch and pectin on the growth of *S. epidermidis* bacteria. The graph demonstrates that at 3rd hour of incubation, pectin had the lowest OD600 value when compared to the comparative compound, inulin, and control. Pectin had an OD600 value equivalent to the control and lower than starch and inulin after 24 hours of incubation. At the 3rd and 24th hours of incubation, starch had the highest OD600 value when compared to glucose and inulin and controls. Overall, the number of *S. epidermidis* treated with pectin was the lowest when compared to starch, inulin, and glucose after 3 or 24 hours of incubation. *S. epidermidis* that was treated with starch had the highest number than cultures that were treated with pectin, inulin, and glucose although not statistically significant (p>0,05). The percentage of inhibition was calculated using the OD600 values obtained from the test and control substances. Table 1 shows the percentage inhibition of *P. acnes* and *S. epidermidis*. Starch inhibited *P. acnes* the most at 3 hours compared to pectin, inulin, and glucose, but its inhibition was reduced at 24 hours. Conversely, glucose promotes the development of *P. acnes* at 24 hours. This is most likely due to the fact that *P. acnes* cannot use starch as a carbon source to promote its growth, but can use glucose as one.[15,16]

Pectin inhibits *P. acnes* effectively at the 3rd and 24th hours of incubation. This is consistent with prior research, which found that pectin from tea leaves had antiadhesive effects against *P. acnes* in vitro that lead to suppression of the growth of these bacteria.[17] Although based on the Kruskall-walis test a significant value was obtained > 0.05, so there was no difference in the percentage of inhibition of *S. epidermidis* and *P. acnes* at 24 hours for all groups.

The potential of starch, pectin, and inulin to enhance the development of *S.epidermidis* was demonstrated after 24 hours of incubation with a negative inhibition percentage as a sign. In comparison to glucose, inulin, and

Table 1. Percent Inhibition of the Test Compounds on the Growth of *P. acnes* and *S. epidermidis*

Compound	Percentage of inhibition against *P. acnes* at		Percentage of inhibition against *S. epidermidis* at	
	3 h of incubation	24 h of incubation	3 h of incubation	24 h of incubation
Glucose	13.02 ± 2.16	-24.03 ± 13.39	-5.63 ± 0.88	-120.22 ± 37.09
Inulin	30.07 ± 1,90	-2.13 ± 19.67	18.04 ± 1.47	-54.05 ± 24.20
Ambon banana starch	71.44 ± 0.79	1.62 ± 3.88	61.43 ± 0.81	-184.95 ± 20.76
Ambon banana pectin	58.01 ± 0.76	65.07 ± 0.72	71.53 ± 0.67	-5.80 ± 15.63

The data are presented as mean ± SD (n=2)

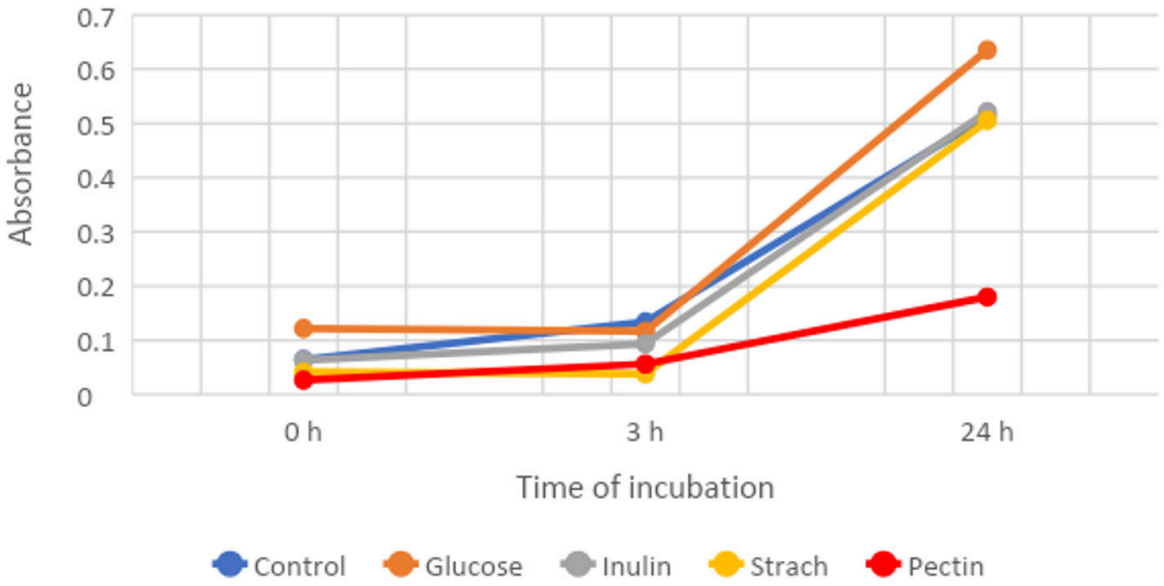

Figure 1. The average OD600 values (n=2) of *P. acnes* Cultures Treated with Starch and Ambon Banana Peel Pectin compared to Glucose, Inulin, and the Control.

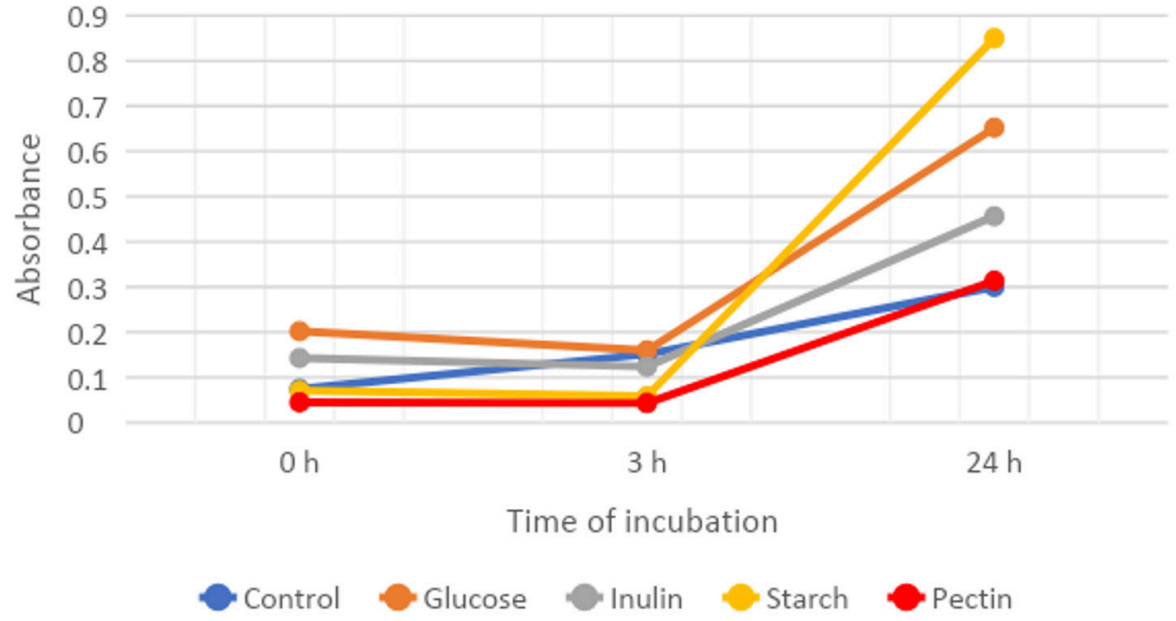

Figure 2. The average OD600 values (n=2) of *S. epidermidis* Cultures Treated with Starch and Ambon Banana Peel Pectin compared to Glucose, Inulin, and the Control.

pectin, starch gives the lowest percentage of inhibition, implying that starch might increase the number of *S. epidermidis* the most. Glucose also demonstrated a negative inhibitory percentage at 24 hours of the incubation period, which was similar to *P. acnes*, implying that glucose can promote the development of both bacteria.

Starch and pectin exhibit a negative inhibition percentage for *S.epidermidis* and a positive inhibition percentage for *P.acnes* indicating that these substances promote *S. epidermidis* development but inhibit *P.acnes* growth. The ability to selectively increase the growth of beneficial bacteria is called prebiotics. The prebiotic activities of Ambon banana peel pectin is comparable to that of tea leaves pectin, which has also been shown to have a selective effect in enhancing *S.epidermidis* and decreasing *P.acnes*.[17] In terms of percentage inhibition, starch, and pectin have a greater potential to enhance the number of *S.epidermidis* while decreasing the number of *P.acnes* than inulin.

Prebiotics can be administered orally or topically to balance the composition of opportunistic microbiota and minimize inflammatory reactions in treating acne vulgaris (acne).[4] Antibiotics, stress, and diet can all affect changes in microbiota makeup in the skin.[18] Alteration in the microbiota composition, such as an increase in the number of *P. acnes*, might increase the likelihood of getting acne.[19]

In acne-prone skin, the increased number of S. epidermidis caused by prebiotic treatment can restrict the development of *P. acnes* through various predicted mechanisms. S. epidermidis can create succinic acid, which inhibits *P. acnes* bacterial growth.[20] *S. epidermidis* is also thought to create a polymorphic toxin that inhibits *P. acnes* growth. Christensen et al. (2016). *S. epidermidis* can also create lipoteichoic acid, which can diminish *P. acnes*-induced inflammation.[21,22]

The anti-inflammatory action of kepok banana peel extract in the treatment of acne has been researched, and the substances considered to be responsible for this effect are ascorbic acid, carotene, cyanidin, trigonelline, isovanillic acid, and ferulic acid.[23] The prebiotic effect of banana peel starch and pectin on skin microbiota has not been extensively studied. Still, starch has been studied as an additive in cosmetic formulas used to treat acne caused by *P. acnes*.[24–26]

Pectin has been researched as an anti-acne excipient and has been shown to boost the anti-acne impact of the active ingredients used in pharmaceutical preparations.[27,28] The prebiotic impact may contribute to the preparation's anti-acne efficacy. Based on the findings of this study's prebiotic effects of starch and pectin from Ambon banana peels, these two compounds have the potential to be developed as excipients that strengthen the effects of active ingredients in acne treatment formulations.

Conclusion

Ambon banana peel starch and pectin have been shown to have prebiotic potential because they inhibited P. acnes growth with a percentage of inhibition of 1.62% and 65.07%, respectively, and increased the growth of S. epidermidis with a percentage of inhibition of -184.95% and -5.80%, respectively.

Acknowledgement

We thank to Dieni Mardliyani for her contribution in conducting experiments in the laboratory. We also thank Hendra for his assistance in analyzing the data statistically.

Funding
We gratefully thank to Lembaga Penelitian dan Pengabdian Kepada Masyarakat (LPPM) for providing fund in this project (Contract Number: 009/B.04/LPPM/I/2023).

Conflict of Interest
None declared

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