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Study on the Antibacterial Effect and Mechanism of Pectin and Its Enzymatic Hydrolysates

Gerald Y. Luo

Beijing Zhongguancun High School, Beijing, China
gojerry2026@gmail.com

Abstract. To examine whether specific pectins or their enzymatic hydrolysates exhibit antibacterial activity and what governs their efficacy, this study investigated the antibacterial effects and mechanism of apple pectin, citrus pectin, and their enzymatic hydrolysates against Escherichia coli, Staphylococcus aureus, and lactic acid bacteria. Antibacterial activity was quantified by measuring inhibition zones in bacterial cultures. The underlying mechanism was probed via electrical conductivity assays, indicating membrane disruption potential. Results revealed distinct activity profiles: citrus pectin hydrolysate and native apple pectin demonstrated significant antibacterial effects. Conversely, native citrus pectin and apple pectin hydrolysate showed no substantial activity. These findings highlight that antibacterial efficacy depends critically on both the pectin source (apple vs. citrus) and its enzymatic modification status (native vs. hydrolysate), suggesting structural specificity in their interaction with bacterial membranes as a key mechanistic factor.

Keywords: Pectin, Enzymatic hydrolysis, Antibacterial activity

1. Introduction

Plant foods are rich in dietary fiber, of which pectin is an important component. In daily life, traditional experience shows that some fruits and vegetables have antidiarrheal effects, and clinical data show that pectin in fruits and vegetables can improve the efficiency of gastric cancer treatment by forming a protective film on the gastric mucosa. At the same time, dietary fiber will affect the intestinal environment and promote the proliferation of beneficial intestinal bacteria. At present, although certain research results have been achieved in the field of pectin at home and abroad, such as some studies focusing on the effects of pectin on intestinal flora, and some explorations on pectin enzymatic hydrolysates. However, as far as China is concerned, there are relatively few literatures on the analysis of the antidiarrheal mechanism of pectin. In addition, in terms of the antibacterial effect of pectin and its enzymatic hydrolysates, although it is known that it has an antibacterial effect, the specific antibacterial mechanism and the differences in the antibacterial effects of pectin from different sources (such as apples, citrus, etc.) and related influencing factors still need to be further clarified and improved. In view of this, this study aims to further explore the antibacterial effect of pectin and its enzymatic hydrolysis products through a series of experiments, analyze its effects on different intestinal bacteria (such as Escherichia coli, Staphylococcus aureus, and lactic acid bacteria), and deeply explore its antibacterial mechanism. It is hoped that it can fill the gaps in

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existing research in these aspects and provide a theoretical basis and practical reference for the scientific and rational application of pectin in food, medicine and other related fields.

2. Materials and methods

2.1. Reagents and strains

Apple pectin, citrus pectin, disodium hydrogen phosphate, citric acid, pectinase, sterile water, agar medium etc. as well as different intestinal bacteria: Escherichia coli, Staphylococcus aureus, and lactic acid bacteria were ordered and prepared as instructed.

2.2. Buffers preparation

Weigh 14.19 g of disodium hydrogen phosphate and 19.2 g of citric acid and dissolve into one liter of aqueous solution to prepare a 0.1 mol/L disodium hydrogen phosphate-citric acid buffer solution, adjust pH to 3.5.

1.5 g of apple pectin and citrus pectin were weighed and dissolved in 150 mL of 0.1 mol/L disodium hydrogen phosphate-citric acid buffer (pH=3.5), and slightly heated (70-80°C) to completely dissolve the pectin [1].

Weigh 0.1 g pectinase accurately, dissolve it in 10 mL 0.1 mol/L disodium hydrogen phosphate-citric acid buffer, filter it, add it to 100 mL pectin solution, mix it evenly, and place it in a 50°C water bath for enzymatic reaction. Take a sample of 10 mL at 100 min and heat it (100°C) to inactivate the enzyme. Finally, bottle it, number it, and sterilize it for later use.

2.3. Antibacterial test of pectin and pectin hydrolysate on the tested bacteria

Immerse the 6 mm diameter dry heat sterilized filter paper in each pectin and its enzymatic hydrolyzate solution, soak for 1 min, take out and dry slightly under sterile conditions; after activating each test strain by slant culture, scrape the test strain with a sampler and add sterile water to make a bacterial solution with a bacterial count of 10^5~10^8/mL, and then take 0.1 mL of each and evenly spread it on the nutrient agar medium plate; then place the dried filter paper at equal distances on the plate coated with bacterial solution (4 pieces/plate, with only bacterial solution as control), mark it, invert it in a constant temperature incubator (37 ℃) for 24 h, and observe the results; measure the diameter of the inhibition zone and calculate the average value and record it in the table. Make 3 parallel samples for each experimental group.

2.4. Measurement of relative conductivity of mixed bacterial culture with solution of pectin and pectin hydrolysate

Escherichia coli, selected based on its significant sensitivity in prior antibacterial assays, was cultured in peptone medium at 37 °C for 12 h, harvested, and adjusted to 10^6–10^8 CFU/mL in sterile saline. Aliquots (10 mL) of this suspension were separately mixed with 10 mL of either apple pectin solution, apple pectin enzymatic hydrolysate solution, or sterile buffer (control), followed by incubation in a 37 °C water bath. After 100 min, a 10 mL sample was withdrawn from each mixture and immediately heat-inactivated at 100 °C for 5 min to terminate biological activity (killing bacteria and denaturing residual enzyme in the hydrolysate sample). The electrical conductivity of each inactivated sample was measured in triplicate using a calibrated conductivity meter at ambient temperature (~25 °C), and the mean value was calculated. The relative electrical conductivity was

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determined according to Wang et al. [2] to assess membrane permeability as an indicator of antibacterial mechanism.

3. Results

3.1. Determination of antibacterial effects of different pectins and their enzymatic hydrolysates

After the plates with different pectins and their hydrolysates were inoculated with the tested bacteria, they were placed in a constant temperature incubator for 24 h, and the antibacterial effect of the pectin hydrolysate was observed. The results are shown in Table 1.

According to the data in Table 1, there was no antibacterial zone in the control group; apple pectin had a strong inhibitory effect on Escherichia coli, and the diameter of the antibacterial zone was more than 10 mm, which was very different from the control group; while the antibacterial effect of apple pectin hydrolysate was not obvious, and the diameter of the antibacterial zone was 6.0-7.5 mm, which was not significantly different from the control group; citrus pectin hydrolysate had a certain inhibitory effect on Staphylococcus aureus, and the diameter of the antibacterial zone was 7.5-9.0 mm, which was very different from the control group, but the antibacterial effect on Escherichia coli was weak.

Table 1: Diameter of inhibition zone of different antibacterial substances

Antibacterial substances	Escherichia coli	Staphylococcus aureus	Lactic acid bacteria
apple pectin	++++	+	++
apple pectin hydrolysate	+	+	+
citrus pectin	-	-	-
citrus pectin hydrolysate	-	++	-
control group	-	-	-

Note: "-" indicates no inhibition zone, "+" indicates an inhibition zone diameter of 6.0-7.5 mm, "++" indicates an inhibition zone diameter of 7.5-9.0 mm, and "+++" indicates an inhibition zone diameter of more than 10 mm.

Based on literature review [1,3], we found that the antibacterial effects of pectin and its enzymatic hydrolysates are mainly due to the galacturonic acid and polygalacturonic acid contained in pectin. Therefore, the antibacterial effects of apple pectin, orange peel pectin and their enzymatic hydrolysates are different and are affected by their molecular weight. At the same time, different molecular weights of polygalacturonic acid can inhibit different bacterial species [4]. This makes apple pectin have the best antibacterial effect on Escherichia coli, and orange peel pectin enzymatic hydrolysate has the best antibacterial effect on Staphylococcus aureus.

3.2. Study on pectin and its antibacterial mechanism

We studied the antibacterial mechanism of pectin by measuring the conductivity of pectin (pectin hydrolysate) after mixed bacterial culture with various solution. The data are shown in the Table 2.

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Table 2: Determination of conductivity of different mixed solutions (unit: ms/cm)

Group	Mixed solutions	1st	2nd	3rd	Average
1	E. coli + apple pectin mixture	7.83	7.93	7.98	7.91
2	E. coli + apple pectin hydrolysate mixture	12.66	12.66	12.59	12.64
3	E. coli culture	20.2	21.2	21.4	20.9
4	E. coli + Na_2HPO_4-$C_6H_8O_7$ buffer	7.63	7.54	7.49	7.55

Note: The greater the conductivity of a liquid, the better the liquid's ability to conduct electricity.

As shown in table 2, group 1, 2, and 3 were treated as experimental groups, and group 4 was set as the control group. By comparing the results of groups 2 and 4, we found that the conductivity of the mixed solution of pectin hydrolysate and Escherichia coli was much higher than that of the control group, so it can be judged that the antibacterial mechanism of polygalacturonic acid is to destroy the bacterial cell membrane, making the bacterial cell structure incomplete and thus achieving the antibacterial effect; and by comparing the experiments of groups 1 and 4, it was shown that the conductivity of the two mixed solutions was not much different, but in the experiment of 3.1 "Determination of the antibacterial effect of different pectins and their hydrolysates", it was found that apple pectin had an antibacterial effect, so it can be judged that the antibacterial effect of apple pectin is not achieved by destroying the bacterial cell membrane.

4. Conclusion and perspective

4.1. Conclusion

Through a series of experimental studies, we have a clearer understanding of the antibacterial effect and mechanism of pectin and its hydrolysates. In terms of antibacterial effect, pectin and its hydrolysates from different sources show different performances. For example, apple pectin has a strong inhibitory effect on Escherichia coli, while the antibacterial effect of apple pectin hydrolysate is relatively insignificant; citrus pectin hydrolysate has a certain inhibitory effect on Staphylococcus aureus, but the antibacterial effect on other species is weak. This shows that the antibacterial effect of pectin and its hydrolysates varies due to factors such as the source of the raw materials and whether it has been enzymatically hydrolyzed. Its inhibitory ability to different species of bacteria is also different. Pectin has a large molecular weight, and its hydrolysate has a small molecular weight. The antibacterial effect is mainly affected by the molecular weight of galacturonic acid and polygalacturonic acid contained in pectin or its hydrolysate. Different molecular weights of polygalacturonic acid can inhibit different species of bacteria.

In the exploration of the antibacterial mechanism, we conducted research by measuring relative conductivity and other methods. Using Escherichia coli as the test strain, the conductivity of different mixed solutions (such as apple pectin solution mixed with bacterial culture, apple pectin enzymatic hydrolysate mixed with bacterial culture, buffer solution mixed with bacterial culture, etc.) was compared. It was found that the antibacterial effect of apple pectin was not achieved by destroying the bacterial cell membrane, but the antibacterial mechanism of pectin enzymatic hydrolysate was to destroy the bacterial cell membrane, make the bacterial cell structure incomplete, change its permeability and achieve the antibacterial effect.

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In summary, this study clarified that the antibacterial properties of pectin and its enzymatic hydrolysate are affected by multiple factors, and clarified the internal mechanism of its partial antibacterial effect. These conclusions can provide important reference for subsequent research and practice in related food development, intestinal health maintenance, etc.

4.2. Perspective

From the perspective of research significance, the results of this study have a positive impact in many fields. In the field of food science, given that pectin and its enzymatic hydrolysates have antibacterial effects, it can be further explored as a natural food preservative for food preservation, replacing some chemical synthetic preservatives, meeting consumers' demand for natural and healthy food, and improving food quality and safety. In terms of intestinal health, since pectin affects the intestinal environment and interacts with various bacterial species in the intestine, a deep understanding of its antibacterial effects and mechanisms will help develop targeted intestinal microecological regulation products, prevent and improve some intestinal diseases by regulating the balance of intestinal flora, and promote overall human health [5].

However, it cannot be ignored that this study also has certain limitations. On the one hand, although the test strains we selected cover common intestinal-related strains such as Escherichia coli, Staphylococcus aureus, and lactic acid bacteria, the actual human intestinal flora is diverse and the interactions are extremely complex [6]. The range of strains studied is still relatively limited, and may not fully reflect the full antibacterial situation of pectin and its enzymatic hydrolysates in the real intestinal environment. On the other hand, the experimental environment is carried out under relatively ideal in vitro conditions, which is different from the complex physiological environment in the body (such as the influence of digestive juices, intestinal peristalsis, synergistic or antagonistic effects of other food components, etc.), which may cause a certain deviation between the experimental results and the actual application effect. At the same time, our control of the concentration of bacterial solution and pectin solution may cause large errors in the antibacterial experiment, which is also needed to be improved in the future.

Looking forward to the future, there are many directions worthy of further in-depth research. First, expand the range of test strains, include more rare strains in the intestine and bacterial flora combinations under different conditions, and more comprehensively explore the antibacterial performance of pectin and its enzymatic hydrolysates in the complex intestinal flora ecology. Second, carry out more specific microbiological research, we can further explore the mechanism of galacturonic acid breaking the cell membrane, and the characteristics of pectin and its enzymatic hydrolysates at the chemical level. In addition, we can further explore the effects of different processing techniques, modification treatments, etc. on the antibacterial properties of pectin and its enzymatic hydrolysates, tap its greater application potential, and lay a solid foundation for the more scientific, reasonable and efficient application of pectin in multiple industries.

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