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Antibacterial of Endophytic Bacteria from Papaya (*Carica papaya*) Seeds Againts *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Endophytic bacteria were produce antibacterial compounds from various plants has been widely reported as considered efficient in overcoming resistant pathogen infections. This study aims to obtain endophytic bacteria from papaya seeds which can produce antibacterials. Papaya seeds were used to isolate endophytic bacteria, which were then examined for antibacterial activity. The dics diffusion method is used to determine the antibacterial activity's efficacy against Escherichia coli and Staphylococcus aureus. There are nine isolates of endophytic bacteria in all. According to a potency test, the nine isolates exhibit strong antibacterial activity against Escherichia coli and Staphylococcus aureus, respectively. Every isolate has the ability to effectively inhibit Staphylococcus aureus. The only isolates that could suppress Escherichia coli were CPA 5, CPA 6, and CPA 8. Based on their antibacterial activity against Escherichia coli and Staphylococcus aureus, it was determined that the endophytic bacterial isolates from papaya seed have the capacity to generate antibacterials.

Keywords: Antibacterial, endophytic bacteria, papaya, seeds.

Introduction

Bacteria that thrive and proliferate in healthy plant tissue are known as endophytic bacteria. They do not infect or harm their hosts and have a variety of useful properties (Azyenela et al., 2023; Gond et al., 2015). It is possible to isolate endophytic bacteria from fruit, flowers, seeds, leaves, stems, and roots (Afzal et al., 2019; Mahdi et al., 2022). The important roles of Endophytic bacteria in plants such as: triggering plant growth (Plant Growth Promoting) through their ability to absorb and solubilize nutrients, producing antimicrobial components to overcome pathogens that provide protection and supporting plant health, producing various kinds of compounds that are used to control pollution and phytoremediation agents, and produce secondary metabolite components such as antibiotics, antifungals, anti-cancer, antioxidants and bioinsecticides (Hardoim et al., 2015; Pratiwi, et al., 2020; Pratiwi et al., 2024).

The exploration of endophytic bacteria in producing antibiotic compounds from various plants has been widely reported and is considered efficient in overcoming resistant pathogen infections (Afzal et al., 2019; Mahdi et al., 2022). It is well known that natural substances derived from fungi, bacteria, and plants can cure infectious disorders brought on by antibiotic-resistant pathogenic bacteria (Mai et al., 2020). Antibiotic-resistant bacteria can be managed with the use of bioactive substances made by plants (Subramani et al., 2017). The research to obtain new antibiotic agents has focused on endophytic bacteria (Azyenela et al., 2023; Gond et al., 2015).

Several studies related to papaya plants have reported that they contain bioactive compounds and have antibacterial activity. Papaya leaves extracted using ethyl acetate produce antibacterial properties by inhibiting the growth of *Staphylococcus aureus* bacteria (Peter et al., 2014). Papaya peel extract using 96% ethanol successfully demonstrated antibacterial activity against *Propionibacterium acnes* (Torar

et al., 2017). 96% ethanol-based papaya seed extract exhibits antibacterial efficacy against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa (Ariani et al., 2019; Buang et al., 2019). Apart from that, research on endophytic bacteria from Papaya leaves, stems and roots has also found antibacterial activity (Subramani et al., 2017; Ginting et al., 2020; Azyenela et al., 2023). Antibiotics produced from endophytic bacteria are very environmentally friendly, not dangerous to humans and are only toxic to pathogens (Singh et al., 2017; Sharma & Mallubhotla, 2022; Faujiah et al., 2023).

Endophytic bacteria from papaya seeds have not yet been investigated for their antibacterial properties. There is a potential to obtain endophytic bacteria from papaya seed to produce antibacterials similar to the ability produced by the host (papaya plant) according to previous studies. Isolating endophytic bacteria from papaya seeds and assessing their potential antibacterial efficacy against Escherichia coli and Staphyloccus aureus bacteria were the goals of this study. This research is useful in providing new information related to the potential of endophytic bacteria from papaya seeds that produce antibacterial activity and as an alternative to new antibacterial that inhibiting *Staphyloccus aureus* and *Escherichia coli* bacteria.

Materials and Methods

Preparation of Papaya Seeds

Papaya seeds are collected from peeled papaya fruit (Papaya California variety). The seeds are cleaned and washed with sterile distilled water. Next, the seeds are drained and air-dried. Papaya seed samples will be used for the isolation process of endophytic bacteria.

Isolation Of Endophytic Bacteria

Papaya seeds' endophytic bacteria were is olated using a modified version of the methodol ogy used in earlier research (Ginting et al., 2020; Pratiwi, et al., 2020; Semenzato et al., 2022; Azyenela et al., 2023; Pratiwi et al., 2024). Papaya seeds were surface sterilized as a sample. To sterilize the papaya seeds, their surface was submerged in 70% ethanol for two minutes, then sodium hypochlorite 5.25% for one minute, and finally 70% ethanol for three minutes. Following the completion of each step, wash three times for one minute each in sterile distilled water. To confirm that the surface is sterile, checked for feasible microbial growth by inoculating suspension of final washing distilled water on Nutrient Agar media and then incubated for 24 – 48 hours. If there is no microbial growth, this indicates that it is sterile and the papaya seeds can be used for testing.

A 15gram sterile seed samples with 135 mL of 0.85% NaCl is blended to homogeneous with a blender at high speed. The sample suspension is made in diluted using the serial dilution method up to 10^{-6}. A spread plate was used to inoculate each sample suspension (0.1 mL) on Nutrient Agar (NA) media with 50 ppm of Nystatin. The plates were then incubated for 48 hours at 37 °C. After 48 hours, the culture bacteria were observed and characterization. Single colony bacterial isolates were purified using a spread plate method.

Preparation of Endophytic Bacterial Inoculum

Each endophytic bacterial isolates were grown in 50 mL of Nutrient Broth (NB) media at 37 °C for 48 hours as a starter. 5 mL of culture was taken and inoculated into 95 mL of new NB media. Observation of the number of bacterial cells was carried out by counting using a Haemocytometer.

Preparation of Bacterial Test Inoculum

Escherichia coli and Staphylococcus aureus, the test microorganisms, were cultivated in Muller Hinton Agar (MHA) media and incubated for twenty-four hours at 37°C. Measuring the density of each bacterial cell by comparing it using the Mc-Farland method. The number of test pathogenic bacteria is estimated at 10^8 CFU/ml.

Potency Antibacterial Activity Test

The Potency antibacterial activity of endophytic bacteria tests were follows the method from previous studies with the modificatios (Sharma & Mallubhotla, 2022; Azyenela et al., 2023; Faujiah et al., 2023). Potency tests are carried out to select isolates that have the ability to produce antibacterials. Antibacterial activity test were obtain by disc

diffusion method against *Staphylococcus aureus* and *Escherichia coli*.

Each bacterial culture of *Staphylococcus aureus* and *Escherichia coli* (10^8 CFU/mL) were spread plate 0.1 ml on the MHA media. Endophytic bacterial culture grown for 24 hours. For ten minutes, the endophytic bacterial culture was centrifuged at 1.000 rpm and 4°C. After being separated from the pellet, the supernatant was moved to a fresh microtube. Prepare sterile blank discs (size 6 mm) for testing antibacterial activity. Inoculate the supernatant on a sterile disc of 60 µl. Each bacterial test culture (10^8 CFU/mL) was spread on a spread plate of 0.1 ml on the top surface of the Muller Hinton Agar medium. A disc containing the dried supernatant is placed on the top surface of the Mueller Hinton Agar Media which previously contained the test bacterial culture. Incubation at 37 °C for 48 hours.

Observation of the clear zone formed between the disc. The clear zone formed indicates that metabolites from endophytic bacteria have antibacterial activity against bacteria test (*Staphylococcus aureus and Escherichia coli*).

Results and Discussion

Characteristic of Endophytic Bacteria

The results of endophytic bacterial isolation after purification obtained 9 bacterial isolates. Each isolate was identified of characteristic. (Table 1). The morphological characteristics of endophytic bacterial isolates are shown in the following characters: shape, margin, elevation, surface, texture, opacity, colony pigmentation, bacteria shape, and bacteria Gram.

The endophytic bacteria isolate were irregular and circular shape, entire and undulate margin, butyrous (buttery) and mucoid texture, smooth surface, translusent and opaque. The endophytic bacterial isolate were grayish white, white, and milky white for the colony pigmentation. The endophytic bacterial isolate were rod – positive, rod – negative, and coccus - positive. Endophytic bacterial isolates were used for antibiotic activity tests.

Table 1. Characteristic of Endophytic bacteria isolate

Characters	Endophyte Bacteria Isolate								
	CPA 1	**CPA 2**	**CPA 3**	**CPA 4**	**CPA 5**	**CPA 6**	**CPA 7**	**CPA 8**	**CPA 9**
Colony Shape	Irregular	Circular	Irregular	Circular	Irregular	Irregular	Circular	Irregular	Irregular
Colony Margin	Entire	Entire	Undulate	Entire	Entire	Entire	Entire	Undulate	Entire
Colony texture	Butyrous	Butyrous	Butyrous	Mucoid	Mucoid	Butyrous	Butyrous	Mucoid	Butyrous
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Elevation	Flat	Raised	Flat	Flat	Flat	Raised	Raised	Flat	Raised
Opacity	Translusent	Opaque	Opaque	Opaque	Translusent	Opaque	Opaque	Translusent	Opaque
Colony pigmentation	Grayish white	White	White	Grayish white	Grayish white	Milky white	Grayish white	Grayish white	White
Bacteria Shape	Rod	Rod	Rod	Coccus	Rod	Rod	Rod	Rod	Rod
Bacteria Gram	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Positive

Potency Antibacterial Activity

Endophytic bacteria from papaya seeds were tested for their potent antibacterial action against both Gram-positive and Gram-negative bacteria. According to the results of the potency test, endophytic bacteria can stop the growth of other bacteria. The endophytic bacterial isolate is said to possess antibacterial activity if it is able to stop the growth of bacteria. *Staphylococcus aureus* and *Escherichia coli* were used as bacteria in the disk diffusion method to test for antibacterial activity. The clear zone formed by endophyte bacteria as indicator that the bacteria can producing antibacterial activity. The clear zone was measure, then the inhibition zone category is determined. The Category of inhibition zone were shown antibacterial strength (Table 2) (AR et al., 2022; Jamilatun et al., 2020).

The results of the antibacterial activity screening test shown by Table 3 & Table 4. Based on the potency test, different antibacterial activities were obtained from each endophytic bacterial isolate. Some endophytic bacteria can inhibit Gram-positive and inhibit Gram-negative

bacteria. All endophytic bacteria were able to inhibit *Staphylococcus aureus*. Only isolates CPA 5, CPA 6, and CPA 8 were able to inhibit *Escherichia coli.*

Table 2. The inhibition zone categories

Inhibition zone diameter (mm)	Category
>20	Very strong (Very sensitive)
10 – 20	Strong (Sensitive)
5 - 10	Intermediate
< 5	Weak
0	Resistant

Table 3. Antibacterial activity of endophytic bacteria from papaya seeds

Isolate Code	Antibacterial activity against bacterial test.	
	Staphylococcus aureus	***Escherichia coli***
CPA 1	+	-
CPA 2	+	-
CPA 3	+	-
CPA 4	+	-
CPA 5	+	+
CPA 6	+	+
CPA 7	+	-
CPA 8	+	+
CPA 9	+	-

The largest inhibition zone was obtained from isolate CPA 9 (11,4 mm) followed by isolate CPA 1 (10.1 mm), CPA 3 (5,1 mm), CPA 2 (5,0 mm) which inhibited *Staphylococcus aureus*, and isolate CPA 5 (5,0 mm) which inhibited *Escherichia coli*. Based on the categories of inhibition zone (Table 2), the antibacterial activity strength of endophytic bacterial isolates is classified as strong (sensitive), intermediate, weak, and resistant.

The CPA 1 and CPA 9 isolate are strong (sensitive) to inhibit against *Staphylococcus aureus*. The CPA 3 and CPA 2 isolate are intermediate to inhibit against *Staphylococcus aureus*. The CPA 4, CPA 5, CPA 6, CPA 7, and CPA 8 isolate are weak to inhibit against *Staphylococcus aureus respectively.* The CPA 5 isolate are intermediate to inhibit against *Escherichia coli*. The CPA 6 and CPA 8 are weak to inhibit against *Escherichia coli*. Meanwhile, CPA 1, CPA 2, CPA 3, CPA 4, CPA 7, and CPA 9 isolate are resistant to inhibit against *Escherichia coli* respectively.

Table 4. Inhibition zone of antibacterial activity by endophytic bacteria from papaya seeds

Isolate Code	Inhibition Zone (mm)	
	Staphylococcus aureus	***Escherichia coli***
CPA 1	10,2 ± 6,45	0
CPA 2	5,0 ± 3,11	0
CPA 3	5,1 ± 0,04	0
CPA 4	2,6 ± 1,52	0
CPA 5	1,7 ± 0,98	5,0± 1,05
CPA 6	3,58 ±2,09	3,0 ± 2,43
CPA 7	3,6 ± 0,85	0
CPA 8	2,0± 1,80	3,25 ± 1,88
CPA 9	11,4 ± 6,73	0

Discussion

Approximately nine endophytic bacteria were identified from papaya seeds. There were not many endophytic bacteria found in papaya seeds overall. Small amounts of bacteria have also been found in plant parts such flowers, fruits, and seeds (Truyens et al., 2015). Liu et al. (2017) state that a number of variables, such as plant species, genotypes, organs, age, developmental phases, and canopy types, might influence the makeup of endophytic bacteria. Different parts of plant organs cause different bacterial community associations, thus affecting their respective composition and diversity. The microbiome in the endosphere is less diverse than in the rhizosphere and soil. It was concluded that plant parts (organs) affect the total of endophytic bacteria.

Based on results, it is known that there are differences in antibacterial activity produced by endophytic bacterial isolates. *Staphylococcus aureus* is bactericidal to all endophytic bacteria from papaya seeds, whereas certain isolates are bacteriostatic to *Escherichia coli*. The findings demonstrated that endophytic bacteria's antibacterial properties were more effective against Gram-positive bacteria. Gram-positive *Staphylococcus aureus* bacteria are more susceptible to antibacterial agents. Gram-negative *Escherichia coli* bacteria are more resilient to antibacterial agents.

The cell wall structure in bacteria plays an important role in maintaining cell shape and providing a barrier in controlling the transport of water, ions, nutrients, and other molecules such as antibiotics that target bacterial cell wall components. Gram-positive and Gram-negative bacteria differ in the structure of their cell walls, which affects how well they are susceptible to antibacterials. It was discovered that Gram-positive bacteria were more susceptible to antibiotic substances than Gram-negative bacteria. It is easier for antibacterial substances to enter the cell and locate targets to target because Gram-positive bacteria have a single-layered cell wall structure made of thick peptidoglycan without an outer membrane.

Gram-negative bacteria are thought to be more resistant to antibacterial agents due to the complexity of their cell walls, which are made up of peptidoglycan, lipopolysidaride (LPS), and an outer membrane. Gram-positive bacteria seem to be more effective against antibacterial agents than Gram-negative bacteria (Vital et al., 2010; Sarkar et al., 2017; Torar et al., 2017; Riu et al., 2022). Although some isolates were known to have weak antibacterial activity against the two test bacteria, endophytic bacterial isolates had the potential to produce antibacterials. Further research needs to be carried out to optimize the antibacterial activity produced by each endophytic bacterial isolate.

Conclusion

The conclusion of this research was that Nine isolates of endophytic bacteria were successfully isolated from papaya seeds. The endophytic bacteria has potential antibacterial activity against *Staphyloccus aureus* and *Escherichia coli* respectively. All endophytic bacterial isolates were able to inhibit *Staphyloccus aureus*, however, only CPA 5, CPA6, and CPA 8 isolates were able to inhibit *Escherichia coli*

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