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ORIGINAL ARTICLE

ISOLATION OF ENDOPHYTIC BACTERIA FROM TAXUS ROOT (*Taxus sumatrana*) AND TEST ITS POTENCY AS PRODUCER OF ANTIMICROBIAL COMPOUNDS

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Abstract Endophytic bacteria have been extensively studied as a source of new antimicrobial compounds. Research into new antimicrobials is important as an alternative to the treatment of resistant microbes. One potential source of antimicrobial compounds is the Taxus (Taxus sumatrana). This study aimed to investigate the antimicrobial activity produced by the endophytic bacteria of the Taxus. Endophytic bacteria were isolated from the roots of Taxsus plants growing in Mt. Singalang, X Koto District, West Sumatra. Bacteria were isolated by inoculating pieces of Taxus root tissue into a PDA medium. Endophytic bacteria were identified macroscopically and microscopically. Antimicrobial activity was tested with the test microbe using the spot test diffusion method. As a result of the research, 23 isolates of endophytic Taxus bacteria were obtained, of which 21 isolates were gram-positive and 2 isolates were gram-negative bacteria. The results of the antimicrobial activity test yielded only 2 isolates of endophytic bacteria with antibacterial activity.

Keywords: Endophytic Bacteria · Taxus · Antimicrobial

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Introduction

Exploration of new antimicrobial sources needs to be done, to overcome the problem of microbial resistance to active compounds that are currently increasing. The use of endophytic bacteria is one alternative that can be done as a source of antimicrobial active compounds. Endophytic bacteria are bacteria that live in plant tissues and have a mutually symbiotic symbiosis with their host (Kumala et al. 2008). Endophytic bacteria are capable of producing secondary metabolites that are equal to or better than their hosts (Pasappa et al. 2022).

One of the potential endophytic bacteria to be developed is the isolation result of Taxus plants (Taxus sumatrana). Taxus plant is one of the medicinal plants that are widely explored (Adhikari et al. 2018). Research conducted by Iszkuło et al (2013) found several active compounds in Taxus, including flavonoids, phenolics, lignans, taxumairol Q, 13-O-acetylwalifoliol, and tasumatrols E, F, and G, 10-deacetylbaccatin III, and baccatin III. This active compound has been tested for its ability as an anticancer, but not many studies have tested its ability as an antimicrobial.

In general, some active compounds found in Taxus plants are potential compounds as antimicrobial compounds. Research conducted by Badriah et al (2022) proved that flavonoid compounds in plants Ipomoea Batatas L. can inhibit the growth of Escherichia coli and Staphylococcus aureus. Next, the tests performed by Hardiansi et al (2020) against Staphylococcus aureus and Candida albicans proved phenolic compounds from Acorus calamus extract can inhibit the growth of test microbes.

Based on this background, a study was conducted that aimed to test the potential of Taxus plants as producers of antimicrobial compounds.

Material and Methods

Taxus root samples

Taxus roots come from Desa Pandai Sikek, X Koto District, Tanah Datar Regency, West Sumatra. The root part that was sampled in this study was the cortex. The roots of Taxus used are those in position: roots attached to the stone, roots that are below the soil surface, and roots that are above the soil surface.

Surface Sterilization of Taxus Roots

Sterilization of the root surface of Taxus is carried out using a 1% hypochlorite solution. Root samples (1x1 cm) that have been washed and soaked in several solutions: 70% alcohol for 1 minute, hypochlorite 1% for 2 minutes, and 70% alcohol for 30 seconds based on advice from Yandila et al (2018). For every change of solution, the sample is washed using sterile aquades and dried using paper towels (Handayani et al. 2020).

Isolation and Purification of Endophytic Bacteria

Root tissue that has sterilized its surface is inoculated on the medium of Nutrient Agar (NA) (Djamaan et al. 2017). The medium containing pieces of Taxus root tissue is incubated for 24-48 hours at room temperature. The bacteria growing around the tissue are gradually purified. The bacterial purification technique uses the streak plate method. Pure bacterial colonies are stored in the form of culture stocks on an inclined NA medium (Putri et al. 2018).

Identification of Endophytic Bacteria

Identification of endophytic bacteria is carried out macroscopically and microscopically. Macroscopic observations take the form of visualization of the morphology of a single colony of bacteria. The morphological observations observed are the shape, color, edges, and elevation of the bacterial colony. Each colony that has a different morphology is photographed for documentation.

Microscopic observations were made by the Gram staining technique according to Afifah et al (2018). The stained bacteria are observed under a microscope to see the shape of the cells and the type of gram (Pelczar 2019).

Antimicrobial Activity Test

Antimicrobial potency tests are carried out using the point inoculation method. Antimicrobial activity was tested on test microbes (S. aureus, E. coli, and C. albicans). Each test microbial suspension (McFarland equivalent turbidity 0.5) was inoculated into an NA medium (Yahya et al. 2017). Next, endophytic bacteria are inoculated into the medium using the point inoculation method. The culture is inoculated for 1x24 hours (for bacterial test microbes) or 2x24 hours (for fungal test microbes). The clear zone formed around endophytic bacteria was observed and measured using a caliper. Antimicrobial activity is determined based on the inhibitory zone according to the formula put forward by Hudzicki (2009) as follows:

d = average dB – average dA

where :

d = diameter of the inhibitory zone (cm)

dA = diameter of the bacterial colony zone (cm)

dB = clear zone diameter (cm).

Results and Discussion

Macroscopic Observation

This study succeeded in isolating endophytic bacteria from Taxus. Based on morphological observations, the isolation of bacteria managed to obtain several different colony forms. The colonies of endophytic bacteria that were successfully isolated can be seen in Table 1. Based on Table 1, it is known that this type of isolate in root tissue is most commonly found in roots below the soil surface (10 isolates). While the least found in roots attached to stones (5 isolates).

Table 1. Results of morphological observations on endophytic bacteria colonies of Taxus roots

Isolate code	Morphological identification	Isolate code	Morphological identification	Isolate code	Morphological identification
T.ATB1	Color : milky white Edge : entire Elevation : convex Form : circular Size : medium	ATA 1 (1)	Color : White Edge : undulate Elevation : raised Form : spindle Size : medium	ATA1 (2)	Color : milky white Edge : undulate Elevation : raised Form : circular Size : small
T.ATB2	Color : White Edge : undulate Elevation : raised Form : spindle Size : medium	ATA1 (3)	Color : milky white Edge : entire Elevation : raised Form : circular Size : small	ATA1 (6)	Color : milky white Edge : entire Elevation : raised Form : circular Size : large
ATA1 (7)	Color : milky white Edge : undulate Elevation : flat Form : spindle Size : medium	ATA1 (8)	Color : milky white Edge : undulate Elevation : flat Form : spindle Size : small	ATA1 (9)	Color : milky white Edge : undulate Elevation : flat Form : circular Size : medium
ATA1(10)	Color : milky white Edge : undulate Elevation : flat Form : irregular Size : medium	ABA1 (1)	Color : milky white Edge : filamentous Elevation : raised Form : spindle Size : medium	ABA1 (2)	Color : milky white Edge : lobate Elevation : raised Form : spindle Size : large
ABA1 (3)	Color : milky white Edge : lobate Elevation : raised Form : circular Size : medium	ABA1 (5)	Color : milky white Edge : entire Elevation : raised Form : spindle Size : medium	ABA1 (7)	Color : milky white Edge : entire Elevation : raised Form : circular Size : small
ABA1 (8)	Color : milky white Edge : entire Elevation : raised Form : circular Size : large	ABA1 (9)	Color : milky white Edge : undulate Elevation : raised Form : circular Size : large	ABA1 (10)	Color : milky white Edge : entire Elevation : raised Form : spindle Size : small
AAB1 (1)	Color : milky white Edge : entire Elevation : raised Form : circular Size : medium	AAB1 (2)	Color : milky white Edge : entire Elevation : flat Form : circular Size : large	AAB1 (3)	Color : milky white Edge : entire Elevation : convex Form : circular Size : medium
AAB1 (4)	Color : milky white Edge : entire Elevation : convex Form : circular Size : small	AAB1 (5)	Color : milky white Edge : entire Convex elevation Form : irregular Size : small		

*Note : *T.ATB and ATA (roots below ground level), ABA (roots on rock surface), and AAB (roots above ground)*

The difference in the number of isolates isolated from the root accompaniment of Taxus can be influenced by several factors. According to Marwan et al (2011), the density of endophytic bacteria in plant tissue depends on the type of plant, plant age, tissue type (roots, stems, and leaves), habitat, and environmental factors. Research conducted by Sulistiyani and Dinihari (2019), concluded that endophytic bacterial populations are found more in the roots or rhizomes, rather than parts of other plant tissues such as stems and leaves.

Microscopic Observation

The results of microscopic observations obtained 21 isolates of gram-positive bacteria and 2 isolates of gram-negative bacteria (out of a total of 23 isolates). Most of the endophytic bacteria isolated were coccus (20 isolates), and only 3 bacterial isolates were bacill. The cell shape and gram type of the Taxus bacteria isolate can be seen in Table 2.

Table 2. Microscopic observations of Taxus root isolates

Isolate Name	Microscopic Observation		Isolate Name	Microscopic Observation	
	Cell shape	Grams		Cell shape	Grams
T.ATB1	***Coccus***	**Negative (-)**	ABA1 (3)	***Coccus***	**Positive (+)**
T.ATB2	***Coccus***	**Negative (-)**	ABA1 (5)	***Coccus***	**Positive (+)**
ATA1 (1)	***Coccus***	**Positive (+)**	ABA1 (7)	***Coccus***	**Positive (+)**
ATA1 (2)	***Coccus***	**Positive (+)**	ABA1 (8)	***Coccus***	**Positive (+)**
ATA1 (3)	***Coccus***	**Positive (+)**	ABA1 (9)	***Coccus***	**Positive (+)**
ATA1 (6)	***Coccus***	**Positive (+)**	ABA1 (10)	***Coccus***	**Positive (+)**
ATA1 (7)	***Coccus***	**Positive (+)**	AAB1 (1)	***Bacill***	**Positive (+)**
ATA1 (8)	***Coccus***	**Positive (+)**	AAB1 (2)	***Coccus***	**Positive (+)**
ATA1 (9)	***Coccus***	**Positive (+)**	AAB1 (3)	***Coccus***	**Positive (+)**
ATA1 (10)	***Coccus***	**Positive (+)**	AAB1 4)	***Bacill***	**Positive (+)**
ABA1 (1)	***Coccus***	**Positive (+)**	AAB1 (5)	***Bacill***	**Positive (+)**
ABA1 (2)	***Coccus***	**Positive (+)**			

Antimicrobial Activity Test

Based on the results of the study, it is known that most endophytic bacteria isolated from the roots of Taxus do not have antimicrobial activity. The test results of the activity of endophytic bacteria against test microbes are shown in Table 3.

Table 3. Test results of antimicrobial activity of Taxus root

Table 3. Test results of antimicrobial activity of Taxus root

Isolate code	Diameter of the inhibitory zone			Isolate code	Diameter of the inhibitory zone		
	S.aureus	***E.coli***	***C.albicans***		***S.aureus***	***E.coli***	***C.albicans***
T.ATB1 (1)	-	-	-	ATA1 (9)	-	-	-
T.ATB1 (2)	-	-	-	ATA1 (10)	-	-	-
ATA1 (1)	4,2 mm	-	-	ABA1 (1)	-	-	-
ATA1 (2)	-	-	-	ABA1 (2)	-	-	-
ATA1 (3)	3,8 mm	-	-	ABA1 (3)	-	-	-

ATA1 (6)	-	-	-	ABA1 (5)	-	-	-
ATA1 (7)	-	-	-	ABA1 (7)	-	-	-
ATA1 (8)	-	-	-	ABA1 (8)	-	-	-
ABA1 (9)	-	-	-	ABA1 (10)	-	-	-
AAB1 (1)	-	-	-	AAB1 (2)	-	-	-
AAB1 (3)	-	-	-	AAB1 (4)	-	-	-
AAB1 (5)	-	-	-				

Table 3 shows that most isolates of Taxus endophytic bacteria cannot inhibit the growth of test microbes. Only ATA1 (1) and ATA1 (3) isolates can inhibit the growth of S. aureus. The antimicrobial ability of these two isolates is relatively low (based on the magnitude of the resulting inhibitory zone).

In theory, Taxus contains active compounds that are known to be antimicrobial. Among these compounds are flavonoids, phenolics, lignans, and paclitaxel (taxol) (Iszkuło et al. 2013). Based on the data in this study, the active compounds owned by Taxus are less effective in inhibiting the growth of test microbes.

The interaction between the mechanism of action of antimicrobial compounds and the pathogenesis of microorganisms is one of the factors that will determine the activity of active compounds (Pelczar 2019). Antimicrobial activity in inhibiting the growth of test microbes is influenced by several factors, including concentration, the intensity of antimicrobial substances, the number of microbes, temperature, microbial species, organic compounds, and pH (Pelczar 2019). To obtain the best antimicrobial activity, it is necessary to optimize each parameter that affects the production of antimicrobial active compounds by endophytic bacteria (Nofrion et al, 2019 dan Putri et al, 2021). Antimicrobial activity by endophytic bacteria Taxus in the new study tested by point diffusion test. Furthermore, it is necessary to optimize the fermentasi process to find out the best activity that can be produced by these endophytic bacteria.

Conclusion

From the research, 23 isolates of Taxus endophytic bacteria were successfully isolated, with 2 isolates that had low antimicrobial activity: ATA1 (1) and ATA1 (3) isolates

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