

Isolation of Endophytic Microbes Producing Antibiotics and Antifungals from Leaf Onion (*Allium fistulosum* L.)

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ABSTRACT

Endophytic microbes are microorganisms that live within plant tissues without harming their hosts. Some endophytic microbes, including bacteria and fungi, are known to produce bioactive compounds such as antibiotics and antifungals that have the potential to address antimicrobial resistance. The objective of this research is to determine the antibiotic and antifungal potential of endophytic bacteria and fungi isolated from the stems and roots of leeks from Parongpong, West Bandung Regency. This study employs an experimental descriptive design using the disk diffusion method (modified Kirby & Bauer test). Several endophytic microbial isolates from leek stems exhibited antibiotic and antifungal activities, with several isolates exhibiting significant activity. The BB 2 isolate showed an inhibition zone of 17 mm against *Salmonella typhimurium* and 13.5 mm against *Staphylococcus aureus*, along with antifungal activity, demonstrating a 10 mm inhibition zone against *Candida albicans*. Additionally, JB 2 and JB 4 isolates, also from leek stems, exhibited antibiotic potential with an 11 mm inhibition zone against *Salmonella typhimurium*. In conclusion, endophytic microbes from leek stems demonstrate greater potential as antibiotic and antifungal agents with stronger activity compared to those from the roots.

Keywords: Endophilic microbes; leaf onions; antibiotic; antifungal.

INTRODUCTION

The use of traditional medicine in Indonesia is increasingly widespread and has become part of the national culture. One of these medicinal plants is leaf onions (*Allium fistulosum* L.) which thrive in the highlands, including in Parongpong District, West Bandung Regency. This area has volcanic soil conditions with a pH of 5.5-7, rainfall of 2,121 mm, temperature of 15-27°C, and humidity of 80%, making it ideal for cultivating vegetables, including leaf onions (1). Ristiana (2023) and Romadhani et al. (2019) (2) Leaf onions, in highlight that such environmental conditions support the optimal growth of these plants. In addition to being commonly used as a cooking ingredient, leaf onions also serve as a potential source of endophytic microbial isolates, as shown in studies by Fajriyati et al. (2022) and Febriyanto et al. (2019) (3).

These endophytic microbes in leaf onions are known to produce broad-spectrum antibiotic and antifungal compounds, which act as natural therapeutic agents. According to Pasappa et al. (2022) (4) and Naibaho et al. (2022) (5), such bioactive compounds are believed to result from coevolution and genetic transfer from leaf onions, exchange with their host, the leaf onion. In addition, leaf onions contain flavonoids, saponins, tannins, and essential oils, which have been reported to inhibit the growth of pathogenic bacteria, as noted by Mandey et al. (2023) (6) and Pratama & Anggraini (2023) (7). Furthermore, the endophytic microbes associated with leaf

onions have shown potential as natural alternatives to conventional antibiotics and antifungals, offering a promising approach to address resistance in emerging pathogenic bacteria, as highlighted (8).

According to Rahardjo et al. (2017) (9) and Nuryah et al. (2019) (10), *Staphylococcus aureus*, a major cause of skin and soft tissue infections, has shown high levels of antibiotic resistance in several regions of Indonesia, reaching up to 79.5% in East Kalimantan. Makatempuge and Lebang (2023) also reported a high resistance rate in *Salmonella typhimurium*, particularly to oxacillin, with a resistance level of 94.4%, posing significant challenges in infection treatment (11). Furthermore, fungal pathogens such as *Candida albicans* and *Aspergillus flavus* have been identified as resistant to antifungal agents, as noted by Abiyoga et al. (2021) and Fakhurrazi et al. (2016), raising further concerns in clinical settings (12).

Previous studies have demonstrated the potential of endophytic microbes as a source of antimicrobial agents. Pakh et al. (2023) successfully isolated endophytic bacteria from taro stalks that exhibited antibacterial activity (13). Similarly, Aqlinia et al. (2020) isolated endophytic bacteria from various parts of the bangle plant, which were capable of inhibiting the growth of pathogenic bacteria (14). In another study, Angelin et al. (2022) found that endophytic fungi derived from leilem leaves were effective in suppressing the growth of *Escherichia coli* and *Staphylococcus aureus*. These findings suggest that endophytic microbes are capable of secreting bioactive compounds to inhibit the growth of surrounding pathogenic bacteria.

As highlighted by Kusumawati and Bintang (2023), the increasing prevalence of antibiotic resistance underscores the importance of discovering natural antibiotics, particularly those derived from endophytic microbes symbiotically associated with medicinal plants. In response to this issue, the present study aims to isolate endophytic bacteria from leaf onions cultivated in Parongpong, West Bandung Regency, and to evaluate their antibacterial and antifungal potential against *Staphylococcus aureus*, *Salmonella typhimurium*, *Aspergillus flavus*, and *Candida albicans* (15).

METHODS

Tool

The tools used in this study include analytical scales, micropipettes, laminar air flow, incubators, light microscopes, autoclaves, petri dishes, stirring rod, test tube, oven, erlenmeyer flask, and graduated cylinder.

Materials

The materials used are leaf onion stems (*Allium fistulosum* L.) taken from Parongpong, West Bandung Regency as a source of endophytic bacteria, sterile distilled water for sterilization of tools and materials, 70% technical alcohol as an initial disinfectant, and 5,25% NaOCl solution for sterilization of sample surfaces. In addition, media, Nutrient Agar (NA) is used for the growth of endophytic and pathogenic bacteria, while Potato Dextrose Agar (PDA) media is used to grow endophytic and pathogenic fungi, Nutrient Broth (NB) media to make suspensions of pathogenic bacteria and Potato Dextrose Broth (PDB) media to make suspensions of pathogenic fungi. The pathogenic bacteria and fungi tested included *Staphylococcus aureus*. ATCC 25923, *Salmonella typhimurium* ATCC 25241, *Aspergillus flavus* ATCC 9643, and *Candida albicans* ATCC 10231 as target microorganisms in antibacterial and antifungal activity tests.

Isolation of Endophytic Microbes

The plant parts used were fresh leaf onion stems and roots. The stems and roots were first cleaned with running water, then cut into pieces 2-5 cm long. Each piece of leaf onion stem and root was surface sterilized by being

soaked in 70% technical alcohol for 1 minute, followed by soaking in 5.25% NaOCl solution for 5 minutes, and rinsing again with 70% technical alcohol for 30 seconds. After the sterilization process, the leaf onion stem pieces were planted in NA and PDA media to grow endophytic bacteria and fungi. The media that had been inoculated with the samples were then incubated using an incubator at 37°C for 24 hours for endophytic bacteria, while for endophytic fungi, it was incubated at room temperature for 48 hours. The endophytic microbial colonies that grew were purified one by one and rejuvenated on the media to maintain the purity of the culture (16).

Macroscopic and Microscopic Observations of Endophytic Microbe Maintenance Cultures Results

Macroscopic observations were made on bacterial colonies before being subculture on NA media to observe the characteristics of the colonies. Aspects that were observed included colony shape (round, irregular), colony color (white, cream), elevation (flat, protruding), and colony surface texture. Microscopic observations were carried out using the Gram staining method to determine the characteristics of endophytic bacteria and fungi, such as cell shape and Gram staining reactions (positive or negative) to assist in further identification (17).

Antibacterial and Antifungal Activity Test by Diffusion Disc Method (Kirby-Bauer) Modification

One loop of control microbes, consisting of gram-positive bacteria, gram-negative bacteria, and mold and yeast fungi, was taken from the agar stock and grown in 5 ml of sterile liquid media, where Nutrient Broth (NB) was used for bacteria and Potato Dextrose Broth (PDB) for fungi. The inoculated liquid media were then incubated at 37°C for 24 hours for bacteria and at room temperature for 48 hours for fungi. After that, the incubated control microbes were vortexed or homogenized, and 2.5 mL of culture was added to 50 mL of solid media (Nutrient Agar for bacteria and Potato Dextrose Agar for fungi) at a temperature of 35-40°C, where care was taken so that the media was not too hot to avoid microbial death. After being poured into a petri dish with approximately 10 mL, the media was cooled. The endophyte isolate to be tested was placed on a medium that already contained pathogenic bacteria by being inoculated with an ose. To measure the ability of endophytes that have antibacterial or antifungal activity, the clear zones that appeared on the top, bottom, right, and left were measured, and the measurement results were averaged (16).

RESULT AND DISCUSSION

Isolation of Endophytic Microbes

The results of the isolation of endophytic microbes from leaf onions (*Allium fistulosum* L.) are seen in Figure 1. These results show the number of endophytic microbes that were successfully isolated.

Figure 1 shows, isolation of stems and roots of leaf onions on PDA media and NA media incubated at room temperature for 72 hours for fungi and incubated in an incubator for 24 hours for bacteria showed that there were five isolates of endophytic bacteria on stems of leaf onions and four isolates of endophytic bacteria on roots of leaf onions, each of which was coded BB (Stem Bacteria) and BA (Root Bacteria). For endophytic fungi on stems and roots of leaf onions, five isolates were obtained which were coded JB (Stem Fungus) and JA (Root Fungus).

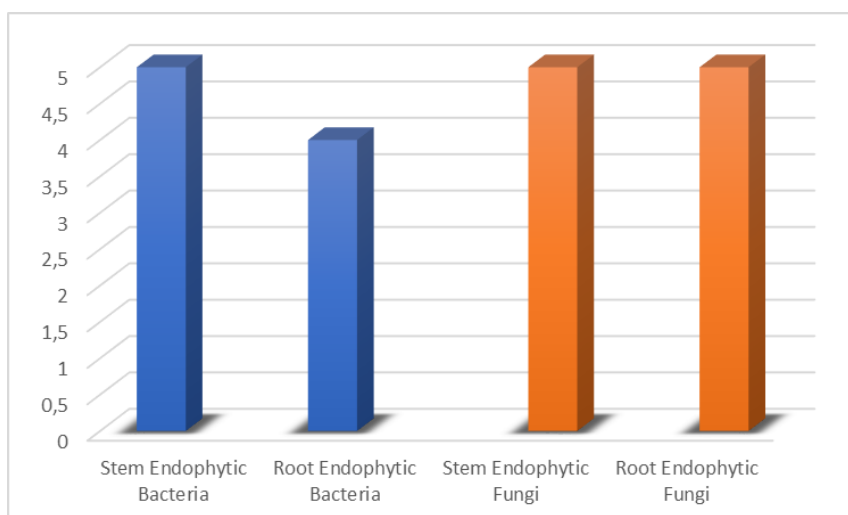


Figure 1. Result of Endophytic Microbe Isolation

Macroscopic and Microscopic Observations of Endophytic Microbes

The results of macroscopic and microscopic observations of endophytic microbes are seen in tables 1, 2, 3 and 4. These results show the macroscopic and microscopic characteristics of the colonies.

Table 1. Results of Macroscopic and Microscopic Observations of Endophytic Bacteria from Scallion Stems at 100x microscope magnification

No	Isolation Code	Colony Morphology (Shape; Color; Elevation; Edge)	Cell Morphology	Gram Staining Result
1	BB 1	Round; White; Convex; Flat	Rod	Purple in a row, Gram positive
2	BB 2	Round; White; Convex; Serrated	Coccus	Purple clustered like grapes, Gram positive
3	BB 3	Round; White; Convex; Flat	Rod	Purple in a row, Gram positive
4	BB 4	Irregular; Creamy white; Convex; Serrated	Rod	Purple in a row, Gram positive
5	BB 5	Round; Creamy white; Convex; Flat	Rod	Purple in a row, Gram positive

As shown in Tabel 1, the results of endophytic bacterial isolation from leaf onion stems on NA media incubated for 24 hours in an incubator, four endophytic bacterial isolates were found with various morphological characteristics, and were given isolate codes BB 1, BB 2, BB 3, BB 4, and BB 5. Macroscopic observations showed that endophytic bacteria from leaf onion stems grown on NA media, with isolate codes BB 1 to BB 5, had morphological differences. Isolates BB 1 and BB 3 showed similarities in shape, color, elevation, edges, and surfaces, while other isolates only had similarities in several characteristics (18). Based on microscopic observations with a light microscope at 100x magnification on the objective lens and gram staining, the five isolates that grew (BB 1 to BB 5) showed round and rod shapes and were purple in color, indicating gram-positive properties(18).

Table 2. Results of Macroscopic and Microscopic Observations of Endophytic Bacteria from Scallion Roots at 100x microscope magnification

No	Isolation Code	Colony Morphology (Shape; Color; Elevation; Edge)	Cell Morphology	Gram Staining Result
1	BA 1	Round; Milky white; Convex; Flat	Rod	Purple single arrangement, Gram positive
2	BA 2	Irregular; Milky white; Convex; Serrated	Rod	Purple single arrangement, Gram positive
3	BA 3	Irregular; Milky white; Convex; Flat	Rod	Purple single arrangement, Gram positive
4	BA 4	Round; Milky white; Convex; Serrated	Rod	Purple single arrangement, Gram positive

As shown in table 2, the results of endophytic bacterial isolation from leaf onion roots on NA media incubated for 24 hours in an incubator, four endophytic bacterial isolates were found with different morphological characteristics, each given the isolate code BA 1, BA 2, BA 3, and BA 4. Macroscopic observations showed that endophytic bacteria from leaf onion stems grown on NA media with isolate codes BA 1, BA 2, BA 3, and BA 4 showed the shape round, and irregular colony shapes, with a milky white color. The edges of the colonies were flat and some were serrated, with convex elevations, and surfaces that varied between smooth and rough. Based on microscopic observations with a light microscope at 100x magnification on the objective lens and gram staining, the four bacterial isolates (BA 1 to BA 4) showed a rod shape and were purple in color, indicating gram-positive properties (19).

Table 3. Results of Macroscopic and Microscopic Observations of Endophytic Fungi from Scallion Stem at 100x microscope magnification

No	Isolation Code	Colony Morphology (Shape; Color; Elevation; Edge)	Cell Morphology	Gram Staining Result
1	JB 1	Round; White-yellow; Convex; Wavy	Round conidiospores; septate hyphae	Red; Mold
2	JB 2	Irregular and spreading; White; Flat; Wavy	Hyphe senocytic (aseptate hyphae)	Red; Mold
3	JB 3	Round; Cream; Flat; Edge flat	Short cylindrical	Purple; Yeast
4	JB 4	Round; White; Convex; Flat	Hyphe senocytic (aseptate hyphae)	Purple; Mold
5	JB 5	White-Yellow; Convex; Flat	Aseptate hyphae	Red; Mold

As shown in table 3, the results of the isolation of leaf onion stems on PDA media incubated for 72 hours in an incubator, five isolates of endophytic fungi were obtained with isolate codes JB 1, JB 2, JB 3, JB 4, and JB 5. Macroscopic observations showed that endophytic fungi from leaf onion stems grown on PDA media with isolate codes JB 1, JB 2, JB 4, and JB 5 had varying characteristics and morphologies and were included in the mold group, characterized by the presence of filaments or mycelium, while isolates with code JB 3 had typical yeast characteristics, which did not have filaments or mycelium (18). Based on microscopic observations using

a light microscope with 100x magnification on the objective lens and gram staining, five isolates (JB 1 to JB 5) were obtained which showed varying morphologies.

Table 4. Results of Macroscopic and Microscopic Observations of Endophytic Fungi from Scallion Roots at 100x microscope magnification

No	Isolation Code	Colony Morphology (Shape; Color; Elevation; Edge)	Cell Morphology	Gram Staining Result
1	JA 1	Round; Yellow; Convex; Serrated	Branched; septate hyphae; genus penicillium; round conidia	Purple; Mold
2	JA 2	Round; White; Convex; Serrated	Branched; aseptate hyphae; genus penicillium; round conidia	Purple; Yeast
3	JA 3	Round; White; Convex; Serrated	Branched; septate hyphae; genus penicillium; round conidia	Purple; Yeast
4	JA 4	Irregular; White; Convex; Serrated	Branched; aseptate hyphae; genus penicillium; round conidia	Purple; Mold
5	JA 5	Round; White Cream; Convex; Flat	Branched; aseptate hyphae; genus penicillium; round conidia	Purple; Mold

As shown in table 4, the results of the isolation of leaf onion stems on PDA media incubated for 72 hours. in the incubator, five isolates of endophytic fungi were obtained with isolate codes JA 1, JA 2 JA 3, JA 4, and JA 5. Macroscopic observations showed that endophytic fungi from leaf onion roots grown on PDA media with isolate codes JA 1, JA 2, JA 4, and JA 5 had varying characteristics and morphologies and were classified as molds, which were characterized by the presence of filaments or mycelium. In contrast, isolates with code JA 3 showed yeast characteristics, which did not have filaments or mycelium (18). Based on microscopic observations using a light microscope with 100x magnification on the objective lens and gram staining, five isolates (JA 1 to JA 5) were obtained which showed varying morphologies.

Inhibitory Test of Endophytic Bacteria in Leaf Onion stems and Roots of Leeks Against *Staphylococcus aureus* ATCC 25923 and *Salmonella typhimurium* ATCC 25241

The results of Endophytic Bacterial Inhibition test on leaf onion stems and roots are seen in Figure 2 and 3.

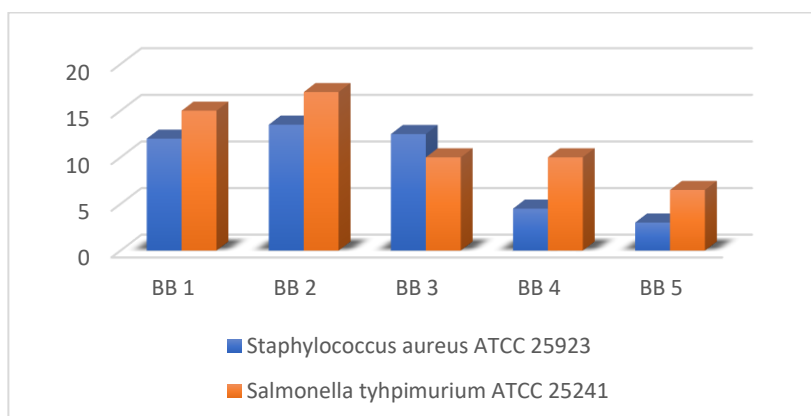


Figure 2. Diagram of the Inhibition Zone Diameter of Endophytic Bacteria of Leaf Onion Stems (*Allium fistulosum* L.) Against *S. aureus* ATCC 25923 and *S. typhimurium* ATCC 25241

Figure 2 shows the results of the inhibition test of endophytic bacteria from leaf onion stems (*Allium fistulosum* L.) on the test microbe *Staphylococcus aureus* ATCC 25923 obtained the results of isolate BB 1 which was 12 mm, isolate BB2 which was 13.5 mm, isolate BB 3 which was 12.5 mm, isolate BB 4 which was 4.5 and isolate BB 5 which was 3 mm. So BB 1, BB 2 and BB 3 are included in the strong category, BB 4 and BB 5 are included in the weak category. While in the test microbe *Salmonella typhimurium* ATCC 25241 on isolate BB 1 which was 15 mm, isolate BB 2 which was 17 mm, isolate BB 3 which was 10 mm, isolate BE 4 which was 10 mm and isolate BB 5 which was 6.5 mm. So it can be stated that BB 1, BB 2, BB 3, BB 4 are included in the strong category and BB 5 is included in the moderate category.

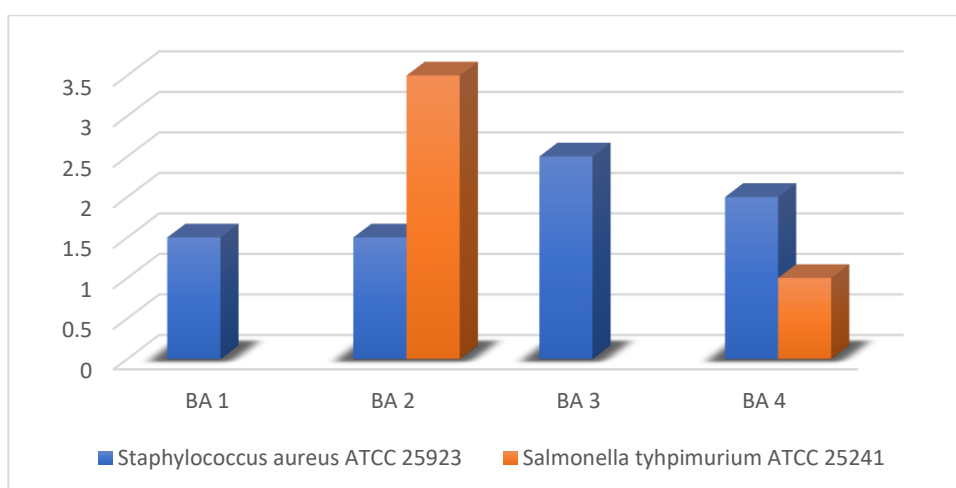


Figure 3. Diagram of the Inhibition Zone Diameter of Endophytic Bacteria in Leaf Onion Roots (*Allium fistulosum* L.) Against *Staphylococcus aureus* ATCC 25923 dan *Salmonella typhimurium* ATCC 25241

Figure 3 shows the results of the microbial test of endophytic bacteria from the roots of leaf onions against *Staphylococcus aureus* ATCC 25923 in isolate BA 1 1.5 mm, in BA 2 namely 1.5 mm, BE 3 namely 2.5 mm, and isolate BA 4, namely 2 mm, and all are included in the weak category. In the test microbe *Salmonella typhimurium* ATCC 25241, the inhibition zone formed was only two isolates, namely in isolate BA 2, namely 3.5 mm, and BA 4, namely 1 mm, included in the weak category. Based on the image, it shows that the effectiveness of endophytic bacteria as antibiotics against *Staphylococcus aureus* ATCC 25923 and *Salmonella typhimurium* ATCC 25241, the results of the endophytic bacterial colony from the roots of leaf onions effectively inhibited the pathogenic bacteria *Staphylococcus aureus* ATCC25923 and *Salmonella typhimurium* ATCC 25241, marked by the formation of a clear zone.

Chloramphenicol was used as a positive control while the negative control used 100% ethanol. The positive control for *Staphylococcus aureus* obtained an inhibition zone of 26.9 mm with a very strong category, while the positive control for *Salmonella* had an inhibition zone of 28.00 mm including a very strong category. Positive controls are used to compare the effects between standard antimicrobial drugs and the samples being tested. Positive controls serve as a reference for the substances being tested, by comparing the diameter of the inhibition zone formed (18). Chloramphenicol was chosen as a positive control because it has broad-spectrum antimicrobial activity, which is able to inhibit the growth of Gram-negative and Gram-positive bacteria. Inhibition of the growth of pathogenic bacteria will increase along with the high concentration of antibacterial compounds contained in the secondary metabolites of these endophytic bacteria. Some isolates may excel in producing secondary metabolites that function as growth regulators, while other isolates may be more effective in producing antibiotics that can fight microbial infections (18).

The formation of a clear zone indicates that the endophytic bacteria have the ability to produce extracellular compounds. According to research the inhibition zone formed can be influenced by various things, including the concentration of the endophytic bacteria tested, temperature and incubation time, and other factors, namely pH and the type of media used. The mechanism of inhibition by endophytic bacteria can occur through the production of antibiotic compounds or competition for space and nutrients between endophytic bacteria and pathogenic bacteria (18). The mechanism of antibacterial compounds is generally carried out by damaging cell walls, changing membrane permeability, disrupting protein synthesis, and inhibiting enzyme activity. These phytochemical compounds play a role in inhibiting bacterial growth. The antimicrobial compounds from leaf onions are effective in inhibiting the growth of pathogens by binding to bacterial cell walls and inhibiting cell wall biosynthesis. The difference in the diameter of the clear zone formed is caused by the difference in the type of antibacterial compounds produced by each endophytic bacterial isolate.

Inhibitory Test of Endophytic Fungi in Leaf Onion stems and Roots of Leeks Against *Aspergillus flavus* ATCC 9643 and *Candida albicans* ATCC 10231

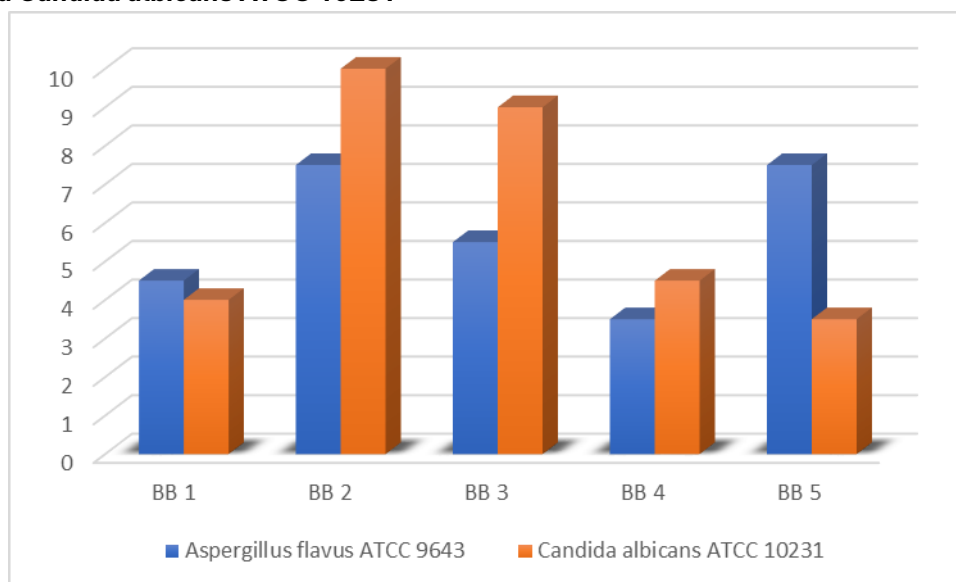


Figure 4. Diagram of the Inhibition Zone Diameter of Endophytic Bacteria in Leaf Onion Stems (*Allium fistulosum* L.) Against *Aspergillus flavus* ATCC 9643 and *Candida albicans* ATCC 10231

The positive control used was ketoconazole, while the negative control used distilled water. The diameter of the inhibition zone in the positive control obtained results of 8 mm in the microbe *Aspergillus flavus* ATCC 9643 and 12.6 mm in *Candida albicans* ATCC 10231. Based on CLSI (Clinical and Laboratory Standard Institute) 2018, the inhibition zone is stated to be sensitive if it is formed by 2-20 mm, intermediate 15-19 mm and resistant ≥ 14 mm. So it can be stated that endophytic bacteria are resistant to *Aspergillus flavus* and *Candida albicans* because the inhibition zone formed is ≤ 14 mm but has the potential for antifungal activity.

The diameter of the inhibition zone in the positive control obtained an average result of 8 mm in the microbe *Aspergillus flavus* ATCC 9643 and an average of 12.6 mm in *Candida albicans* ATCC 10231. The mechanism of action of ketoconazole is by inhibiting the synthesis of ergosterol found in the fungal cell membrane. Ketoconazole acts as a systemic and non-systemic antifungal and is effective against *Candida*, *Aspergillus* and *Histoplasma capsulatum* (18).

Based on the data obtained, the inhibition zone of endophytic bacteria in leaf onion stems against *Aspergillus flavus* ATCC 9643 and *Candida albicans* ATCC 10231 is included in the resistant category. The inhibition zone

formed in the culture of *Aspergillus flavus* ATCC 9643 and *Candida albicans* ATCC 10231 because endophytic bacteria in leaf onion stems contain active compounds that function as antifungals. According to research conducted by. leaf onions contain a number of active compounds, namely flavonoids, saponins, and tannins. The mechanism of flavonoids in inhibiting fungal growth is by disrupting the permeability of the fungal cell membrane. Tannins as antifungals function to inhibit the synthesis of chitin which is needed for the formation of cell walls in fungi, as well as damage the fungal cell membrane, so that fungal growth is inhibited. Saponins as antifungals occur by disrupting the stability of the microbial cell membrane, which ultimately results in cell lysis.

Inhibitory Test of Fungi on Leaf Onion Roots and Roots of Leeks Against *Staphylococcus aureus* ATCC 25923 dan *Salmonella typhimurium* ATCC 25241

The results of Endophytic Fungi Inhibition test on leaf onion stems and roots are seen in figure 5 and 6.

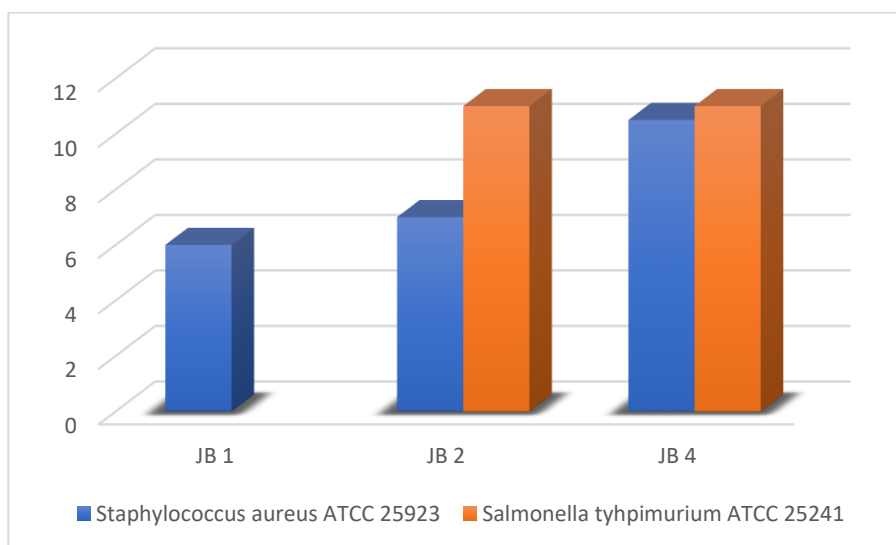


Figure 5. Diagram of the Inhibition Zone Diameter of Endophytic Fungi on Leaf Onion Stems (*Allium fistulosum* L.) Against *Staphylococcus aureus* ATCC 25923 dan *Salmonella typhimurium* ATCC 25241

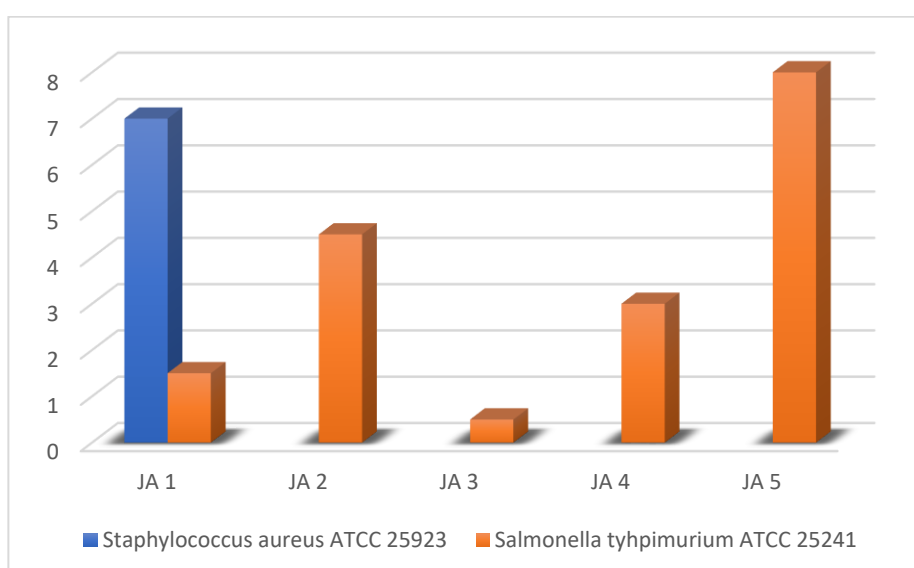


Figure 6. Diagram of the Inhibition Zone Diameter of Endophytic Fungi on Leaf Onion Roots (*Allium fistulosum* L.) Against *Staphylococcus aureus* ATCC 25923 dan *Salmonella typhimurium* ATCC 25241

The study on the antifungal activity of endophytic fungi against *S. aureus* ATCC 25923 and *S. typhimurium* ATCC 25241 was conducted using the modified Disk Diffusion method or modified Kirby-Bauer test, as this method is considered more practical. However, several factors may reduce the effectiveness of this modified Kirby-Bauer test, including temperature, bacterial incubation time, composition of the culture media, antibacterial concentration, and bacterial population.

According to the study by Tangapo et al. (2022), antibiotics can be categorized into four levels based on the diameter of the inhibition zone: very strong (20–35 mm), strong (10–20 mm), moderate (5–10 mm), and weak (≤ 5 mm). The positive control used in this study was chloramphenicol, as it is a broad-spectrum antibiotic and classified as very strong, making it effective in inhibiting the growth of pathogenic bacteria and more efficient than narrow-spectrum antibiotics, which are only effective against either Gram-positive or Gram-negative pathogenic bacteria. The negative control used was distilled water (19).

The positive control on NA media inoculated with *S. aureus* ATCC 25923 was categorized as very strong because the resulting inhibition zone was over 20 mm. Similarly, the positive control on NA media inoculated with *S. typhimurium* ATCC 25241 was also categorized as very strong, as the inhibition zone exceeded 20 mm. No inhibition zone was formed in the negative control (20).

The inhibitory test results of the endophytic fungal isolate (JB) from the stem of *Allium fistulosum* showed that it was capable of inhibiting the growth of the test bacteria *S. aureus* ATCC 25923 and *S. typhimurium* ATCC 25241, as indicated by the formation of inhibition zones around the fungal isolates. The formation of these zones indicates that the endophytic fungi have the ability to produce extracellular antibiotic compounds (21).

Only three out of five fungal isolates were able to inhibit the growth of both pathogenic bacteria. For *S. aureus* ATCC 25923: isolate JB 1 had a 6 mm inhibition zone (moderate category), JB 2 had 7 mm (moderate), and JB 4 had 10.5 mm (strong). For *S. typhimurium* ATCC 25241: JB 2 had 11 mm and JB 4 had 11 mm (both strong) (22).

The inhibition zones of endophytic fungi (JA) from the roots of *Allium fistulosum* L. against *S. typhimurium* ATCC 25241 were as follows: JA 1 = 1.5 mm, JA 2 = 4.5 mm, JA 3 = 2.5 mm, JA 4 = 3 mm, and JA 5 = 8 mm. For *S. aureus* ATCC 25923, only one isolate (JA 1) showed inhibition with a diameter of 7 mm. The variation in inhibition zone diameters was influenced by several factors, including bacterial suspension turbidity, organism sensitivity time, pH, incubation temperature and duration, culture media, agar thickness, and diffusion rate in the agar (22).

Allium fistulosum contains flavonoids, saponins, tannins, and essential oils as secondary metabolites that can inhibit bacterial growth. The antibacterial mechanism of flavonoids involves damaging the bacterial cell membrane by forming complexes with extracellular proteins, leading to the leakage of intracellular compounds and eventual bacterial death. Saponins disrupt bacterial membrane permeability and increase surface tension on the bacterial cell wall, causing extreme stretching, damaging the membrane, and releasing essential bacterial components such as nucleotides, proteins, and nucleic acids, ultimately leading to cell lysis. Tannins inhibit reverse transcriptase and DNA topoisomerase enzymes, preventing bacterial cell formation. Essential oils interact with bacterial cell membranes, affecting cell structure and membrane permeability. This damage continues with ion leakage, followed by cell death (23).

CONCLUSION

Endophytic microbes isolated from the stem of *Allium fistulosum* exhibit greater potential as antibiotic and antifungal agents compared to those from the roots. Isolates from the stem, including BB 2, JB 2, and JB 4, demonstrated significant inhibition zones against pathogenic bacteria such as *Salmonella typhimurium* and *Staphylococcus aureus*, as well as the fungus *Candida albicans*. These findings suggest that stem-derived endophytic microbes of *Allium fistulosum* possess strong potential as natural sources of antibacterial and antifungal agents, which may be utilized in the development of alternative therapies.

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