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Antibacterial Activity of Endophytic Fungi Isolated from Turmeric Plants (*Curcuma longa* L.) Against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Endophytic fungi are fungi that live in a mutualistic relationship with their hosts. They can produce secondary metabolites that are similar to the host plant. Turmeric plants (*Curcuma longa* L.) are highly valued for their medicinal properties and contain active compounds in their rhizomes that act as antibacterials. This study aimed to measure the inhibitory effect of endophytic fungi from turmeric rhizomes against the growth of two bacteria, *Escherichia coli* and *Staphylococcus aureus*. The study involved isolating fungi, observing macroscopic and microscopic morphology, and testing the antibacterial activity of turmeric endophytic fungi against *E. coli* and *S. aureus*. A type of endophytic fungi was successfully isolated from the turmeric plant. The morphological characteristics of the endophytic fungi from turmeric plants include a white colour with a cotton-like texture, umbonate topography, radial lines, and concentric circles. In contrast, the microscopic characteristics include septate hyphae and conidia. Turmeric endophytic fungi were found to inhibit both pathogenic bacteria with inhibition zones of 19.25 mm (strong) against *E. coli* and 13.75 mm (strong) against *S. aureus*. In conclusion, this study successfully isolated endophytic fungi from turmeric plants that can inhibit the growth of *E. coli* and *S. aureus*. However, further identification of the strain and activity tests is necessary to determine the effectiveness of the bioactive compounds in inhibiting the growth of bacteria.

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1. Introduction

Bacteria are becoming resistant to commonly used antibiotics, including *Staphylococcus aureus* and *Escherichia coli* (Aji & Azizah, 2023). *S. aureus* bacteria are Gram-positive bacteria that commonly live on humans' skin and mucous membranes. However, they can sometimes cause infections that range in severity from mild to life-threatening. These infections can affect different body parts, including soft tissues, bones, respiratory organs, and endovascular tissues. They can lead to various symptoms, such as furuncles, impetigo, osteomyelitis, tonsillitis, bronchitis, pneumonia, endocarditis, meningoencephalitis, and sepsis, and are often associated with hospital infections. *S. aureus* strains, particularly the methicillin-resistant ones, are increasingly developing resistance to drugs, making it challenging to treat infections caused by these bacteria. *E. coli* has also developed resistance to antibiotics such as amoxicillin, cefixime, and ciprofloxacin. *E. coli* is a type of bacteria that is rod-shaped and gram-negative. It belongs to the Enterobacteriaceae family and can grow anaerobically or facultatively (Adityawarman et al., 2019). Commonly, *E. coli* can be found in the digestive tract. However, it can become pathogenic and cause harm to both animals and humans in certain circumstances, such as disorders in digestion or immunosuppression in the host (Mundi, 2018).

Alternative treatments that can be used as antibacterials for bacterial infections include using medicinal plants with antibacterial properties, such as readily available and cost-effective turmeric plants. Turmeric, also known as *Curcuma longa* L., is a tropical plant commonly found in various parts of Indonesia. The plant grows annually in clumps and consists of roots, rhizomes, pseudo-stems, midribs, leaves, flower stalks, and flowers. It is a plant used as a spice and adds a bright yellow colour to dishes. It has been utilised for over 2,500 years as a colouring, medicinal, and flavouring ingredient. Turmeric is considered one of the most valuable herbs for human health due to its numerous benefits. Turmeric has long been used to treat wounds, burns, gastrointestinal and liver disorders, respiratory diseases, anorexia, and rheumatism. It has been known to have antiseptic, antibacterial, anti-inflammatory, choleretic, and carminative properties (Nair, 2019). Turmeric contains bioactive compounds that can inhibit the growth of bacteria, including those that cause food poisoning and digestive tract infections, such as *S. aureus* and *E. coli* (Utami & Puspaningtyas, 2013; Adamczak et al., 2020). Furthermore, it is believed that the active compounds are also produced by endophytic bacteria that live on turmeric plants. These endophytic bacteria can produce secondary metabolites, which have antibacterial activity.

Endophytic microorganisms can live and develop in various plant tissues without causing any harm to their host plants (Widowati, 2016; Aji & Sri, 2021). Due to their interaction with host plants, endophytic fungi are believed to co-evolve by sharing beneficial DNA (Tiwari & Bae, 2020). Studies suggest that endophytic fungi exhibit biological activities similar to their host plants. These microorganisms are economically significant because they are easy to grow and have a shorter life cycle than their host plants (Puspitarini & Aji, 2023). They can produce large amounts of bioactive compounds, which may have more significant antibacterial potential than the host plants and can be developed as drugs (Wen et al., 2022; Stelmasiewicz et al., 2023). Therefore, exploring the antibacterial potential of turmeric plant endophytic bacteria against *S. aureus*, one of the pathogens that cause infections, is of great interest to researchers (Astari et al., 2021). This research aims to determine the antibacterial activity of turmeric plant (*C. longa* L.) endophytic bacteria isolates against *S. aureus* and *E. coli* growth. This could provide an alternative solution to help overcome antibiotic resistance, a growing concern in the medical field. Investigating natural sources such as endophytic bacteria from medicinal plants like turmeric offers a promising avenue for discovering new antibiotics that can be more effective against resistant strains (Gond et al., 2015). The isolation and identification of these endophytic bacteria, followed by evaluation of their antibacterial properties, could contribute to developing novel therapeutic agents. Furthermore, understanding how these endophytes inhibit bacterial growth can provide insights into their potential applications in medicine, agriculture, and biotechnology (Tshikhudo et al., 2023). Investigating these endophytes could lead to new antibiotics effective against resistant strains, contributing to novel therapeutic developments and enhancing our understanding of plant-microbe symbiosis.

2. Methods

2.1 Tools and Materials

The study used several tools, including sterile tweezers, sterile scissors, sterile knife, sterile spatula, Bunsen burner, round forceps, petri dish, test tube, test tube rack, drygalski spatula, measuring pipette, pro pipette, incubator, micropipette, microscope slide, coverslip, microscope, dropper pipette, distilled water bottle, and marker. The materials used in the study included Potato Dextrose Agar (PDA) media, chloramphenicol, turmeric plant endophytic fungal isolates, 0.5% NaClO, 70% alcohol, 5.25% Sodium hypochlorite (bayclin), 0.85% NaCl, sterile distilled water, blue pipette tips, 2.5% Sodium hypochlorite, Lactophenol cotton blue, distilled water, Escherichia coli, Staphylococcus aureus, Nutrient Broth (NB) media, yellow pipette tips, and paper discs.

2.2 Sample Preparation

The fresh rhizomes of turmeric plants were labelled and stored using paper clips.

2.3 Fungal Endophyte Isolation

The turmeric samples, consisting of roots, stems, leaves, and rhizomes, were washed with running water. Subsequently, the washed samples were immersed in 70% alcohol for 1 minute, followed by immersion in a 0.5% NaClO solution for 3 minutes, and then dipped in 70% alcohol again for 30 seconds and rinsed with distilled water twice. After sterilization, the sample organs were aseptically cut 1-2 cm long. The cut samples were then inserted into PDA media supplemented with 100µm chloramphenicol and incubated at room temperature for seven days. The pure culture of endophytic fungi was subcultured in PDA media supplemented with chloramphenicol and incubated at room temperature for seven days.

2.4 Macroscopic and Microscopic Characterization

The identification of endophytic fungi was conducted through both macroscopic and microscopic observations. The macroscopic observations examined the fungal colonies on agar media and assessed their color, texture, topography, and radial lines. The microscopic observations involved the preparation of a glass object, labeling it, adding distilled water to the center, aseptically transferring a portion of mold mycelium using a sterilized spatula, spreading it with an ose rod and spatula, adding one drop of Lactophenol cotton blue solution, waiting for one minute for absorption, covering it with a cover glass, and observing it under a microscope.

2.5 Antibacterial activity test

The antibacterial activity test was conducted by pouring 100 µL of *E. coli* and *S. aureus* bacterial isolates into a Petri dish containing NA media and spreading them evenly with a Drygalski spatula. The Petri dishes were divided into four quadrants; then, two fungal colonies were placed in different quadrants. Chloramphenicol, positive control, and distilled water, negative control, were also placed on two different disc papers in the Petri dishes. Subsequently, the culture was incubated for 24 hours at room temperature. Antibacterial activity was assessed by measuring the vertical and horizontal diameters of the inhibition zone formed around the disc papers. Diameter measurements were conducted using a caliper with units of mm. The classification of the Growth Inhibition Zone was based on Astari et al. (2021), where the diameter of the inhibition zone was ≥26.8 mm (very strong), 10.4-26.8 mm (strong); 6.3-10.3 mm (medium), 1.4-6.2 mm (weak); 0 mm (no effect). Data analysis was performed descriptively.

3. Results and Discussion

3.1 Fungal Endophyte Isolation

This study successfully obtained a single endophytic fungal isolate from the rhizome of the turmeric plant. To obtain these fungi, segments of turmeric rhizomes were cultured on PDA (potato dextrose agar) media supplemented with chloramphenicol, a bacteriostatic antibiotic that inhibits microbial growth (Hasanah et al., 2017). Purifying endophytic fungi was done to separate colonies that look different and get pure isolates.

3.2 Macroscopic and Microscopic Characterization

The macroscopic characteristics of endophytic fungi from turmeric plants, as depicted in Figure 1, include colour, texture, radial lines, and concentric circles. These fungi found in turmeric rhizomes are white with a cottony texture, displaying an umbonate or button-shaped topography, along with radial lines and concentric circles. Microscopic observations reveal long hyphae with asepta and conidia. One of these fungi exhibits characteristics such as white mycelium, changing colour and texture like cotton as it ages, umbonate-shaped topography, flat colony elevation, radial lines, and variable colours like white, brownish, and yellowish, as documented by Pakaya et al. (2023). These traits are also present in the isolates successfully obtained in this study.

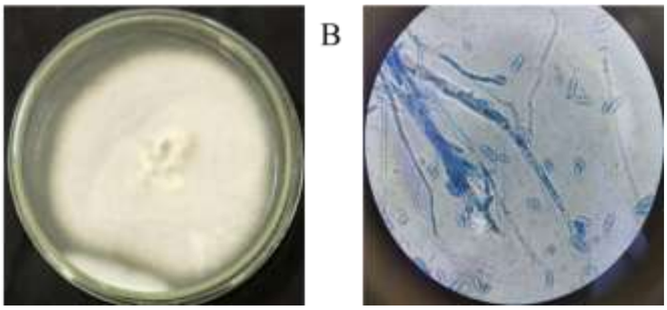

Figure 1. (A) macroscopic and (B) microscopic appearance of turmeric endophytic fungal isolates

3.3. Antibacterial activity test

The antimicrobial activity test is a procedure used to evaluate the antibacterial activity of a substance suspected or known to possess antibacterial properties against a bacterium. The test was conducted on endophytic fungi from turmeric rhizomes using two different pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus*. As shown in Table 1, the results suggest that the inhibitory activity of endophytic fungi from turmeric plants against pathogenic bacteria *E. coli* is strongly inhibitory. This categorisation is based on the average diameter of the clear-coloured area formed around the disc paper. The positive control (chloramphenicol) shows strong inhibitory activity against *E. coli*, with an average diameter of the transparent area around the disc paper of 12.5 mm. In contrast, the negative control shows no clear area was formed, indicating no inhibitory activity against *E. coli*. The average diameter of the clear-coloured area formed around the disc paper by the turmeric endophytic fungal isolate against *E. coli* was 19.25 mm, which indicates strong inhibitory activity against *E. coli*.

Table 1. Antibacterial activity test results of turmeric endophytic fungal isolates

	Escherichia coli		*Staphylococcus aureus*	
	Inhibition zone (mm)	Categories	Inhibition zone (mm)	Categories
Positive control (chloramphenicol)	12.5	Strong	9.5	Medium
Negative control	0	No effect	0	No effect
Turmeric endophytic fungal isolates	19.25	Strong	13.75	Strong

According to Table 1, the endophytic fungi found in turmeric plants have a strong inhibitory effect on the pathogenic bacterium *S. aureus*. This conclusion is based on the average diameter of the transparent area that forms around a paper disc. The transparent area's average diameter in the positive control is 9.5 mm, considered medium. No transparent area was formed in the negative control, indicating no effect against *S. aureus* bacteria. However, the turmeric endophytic fungal isolate shows a clear area's average diameter of 13.75 mm, which is considered strong against *S. aureus* bacteria. According to the antibacterial activity test results, endophytic fungal isolates from turmeric showed antibacterial activity against *E. coli* and *S. aureus*. This was observed through the formation of a clear zone, which is in line with previous studies suggesting that endophytic bacteria found in turmeric plants have the potential to produce substances that act as antibiotics (Sulistiyani et al., 2014). The research findings suggest that fungal endophytes can inhibit *E. coli* and *S. aureus*. This is in line with a study by Al-Fakih and Almaqtri (2019), which found that metabolites from fungi can inhibit both gram-positive and gram-negative bacteria with varying levels of effectiveness. Similar results were also reported by Maliehe et al., (2022), indicating that secondary metabolites from fungal endophytes can inhibit both gram-positive and gram-negative bacteria. The difference in activity between gram-positive and gram-negative bacteria may be attributed to variances in their cell membranes. This supports the findings of Elisha et al., (2017), which showed that extracts are more effective in killing gram-negative than gram-positive bacteria due to their different mechanisms. Additionally, it was noted that these extracts affect the cell membrane and utilise various other mechanisms in fighting gram-negative bacteria (Yan et al., 2024).

Turmeric is referred to as an annual plant that is grown in clumps. Roots, rhizomes, pseudo-stems, midribs, leaves, flower stalks, and flowers constitute turmeric plants. Turmeric is regarded as one of the herbal plants believed to possess many properties. The growth of bacteria causing food poisoning or digestive tract infections (e.g., *E. coli* and *S. aureus*) can be inhibited by turmeric because it contains phenolic compounds that have antimicrobial activity (Utami & Puspaningtyas, 2013). According to Dion et al. (2021), certain types of endophytic fungi, such as Aspergillus, Penicillium, and Fusarium, are commonly found in Curcuma genus plants and can produce antibacterial compounds. The secondary metabolite compounds produced by these endophytic fungi can inhibit the growth of both Gram-positive and Gram-negative bacteria, with a range of 0.9-20 mm (Figure 2). The compounds are effective against various bacteria, including *S. aureus*, *B. subtilis*, *S. agalactiae*, *E. coli* and *S. dysenteriae*. This suggests that the secondary metabolite compounds produced by endophytic fungi associated with Curcuma plants have the potential to be developed into new antibiotics.

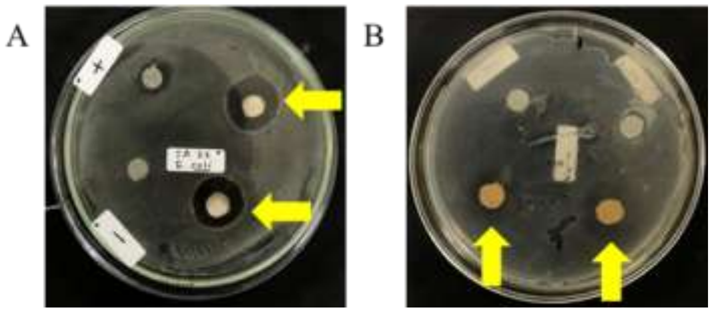

Figure 2. Antibacterial activity test results of turmeric endophytic fungal isolate against (A) *Escherichia coli* and (B) *Staphylococcus aureus*

4. Conclusion

A single strain of endophytic fungi from turmeric was successfully isolated in a study. The test showed that the fungi strongly inhibited the growth of pathogenic bacteria *E. coli* and *S. aureus*. However, further identification of the strain and activity tests are required to determine the effectiveness of the bioactive compounds in inhibiting the growth of these bacteria.

REFERENCES

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020). Curcumin, a Natural Antimicrobial Agent with Strain-Specific Activity. *Pharmaceuticals*, 13(7):153. https://doi.org/10.3390/ph13070153.

Adityawarman, Mahyarudin, Effiana. 2019. Isolasi, Identifikasi dan Aktivitas Antibakteri Bakteri Endofit Daun Pegagan (*Centella asiatica* L.) terhadap *Escherichia coli*. *Jurnal Cerebellum*, 5(4):1569-1582.

Aji, O. R., & Azizah, M. (2023). Antibacterial Activity of Actinomycetes Associated With Clove Plant (*Syzygium aromaticum*) Against *Staphylococcus aureus* and *Escherichia coli*. *Journal of Natural Sciences and Mathematics Research*, 9(2):143-150. https://doi.org/10.21580/jnsmr.2023.9.2.16702.

Aji, O.R., & Sri Wahyuni. (2021). Antagonistic Activity of the Endophy Fungi of Lime Plant (*Citrus aurantifolia*) on *Candida albicans*. *Biosfer: Jurnal Tadris Biologi*, 12(1):65–72. https://doi.org/10.24042/biosfer.v12i1.6367.

Al-Fakih, A. A., & Almaqtri, W. Q. A. (2019). Overview on Antibacterial Metabolites from Terrestrial *Aspergillus* sp. *Mycology*, 10(4):191–209. https://doi.org/10.1080/21501203.2019.1604576.

Astari, S.M., Rialita A., & Mahyarudin 2021. Aktivitas Antibakteri Isolat Bakteri Endofit Tanaman Kunyit (*Curcuma longa* L.) terhadap Pertumbuhan *Staphylococcus aureus*. *Jurnal Fitofarmaka Indonesia*, 8(2):9-16.

Dion, R., Maharani N. A., Akbar M. F., Wijayanti P., & Nurlindasari Y. 2021. Review: Eksplorasi Pemanfaatan Jamur Endofit pada Tanaman *Curcuma* dan *Zingiber* sebagai Penghasil Senyawa Antibakteri. *Jurnal Mikologi Indonesia*, 5(1):16-29.

Elisha, I.L.; Botha, F.S.; McGaw, L.J.; Eloff, J.N. (2017). *The Antibacterial Activity of Extracts of Nine Plant*

Species with Good Activity Against Escherichia coli Against Five Other Bacteria And Cytotoxicity Of Extracts. Med, 17, pp.1–10. BMC Complementary Altern.

Gond, S. K., Verma, V. C., Mishra, A., Kumar, A., & Kharwar, R. N. (2010). *Role of Fungal Endophytes in Plant Protection*. CABI EBooks:183–197. https://doi.org/10.1079/9781845936037.0183.

Hasanah M., Untari, B., & Afrilianti, C. 2017. Analisis Kandungan Senyawa Kloramfenikol pada Sediaan Tetes Mata Sampel Nama Dagang di Kota Palembang dengan Metode Kromatografi Cair Kinerja Tinggi. *Jurnal Ilmiah Bakti Farmasi*, 2(2):47-54.

Maliehe, T. S., Mbambo, M., Nqotheni, M. I., Senzo, N. S., & Shandu, J. S. E. (2022). Antibacterial Effect and Mode of Action of Secondary Metabolites from Fungal Endophyte Associated with *Aloe ferox* Mill. *Microbiology Research*, 13(1):90–101. https://doi.org/10.3390/microbiolres13010007.

Mundi, N. 2018. *Karakterisasi Profil Resistensi Antibiotik pada Escherichia coli yang diisolasi dari Daging Ayam yang dijual di Beberapa Pasar di Surabaya*. Fakultas Kedokteran Hewan. Universitas Airlangga.

Nair, K. P. (2019). *Turmeric (Curcuma longa L.) and Ginger (Zingiber officinale Rosc.)—World's Invaluable Medicinal Spices*. In The Agronomy and Economy of Turmeric and Ginger 1st ed., pp.1–243. Springer Nature.

Pakaya, M. S., Nur A. T., Hamsidar H., Ariani H. H., & Grasela M. 2023. Isolasi, Karakterisasi, dan Uji Antioksidan Fungi Endofit dari Tanaman Batang Kunyit (*Curcuma domestica* Val.,). *Journal Syifa Sciences and Clinical Researc*h, 5(2):220–231.

Puspitarini, S., & Oktira., R. A. (2023). Antagonistic Activities of Endophytic Fungi from Chili Pepper (*Capsicum frutescens* L.) Stems and Leaves against *Colletotrichum* sp. *Journal of Biotechnology and Natural Science*, 3(2):41–46. https://doi.org/10.12928/jbns.v3i2.9736.

Stelmasiewicz, M., Świątek, Ł., Gibbons, S., & Ludwiczuk, A. (2023). Bioactive Compounds Produced by Endophytic Microorganisms Associated with Bryophytes-The "Bryendophytes." *Molecules (Basel, Switzerland)*, 28(7):3246. https://doi.org/10.3390/molecules28073246.

Sulistiyani, R., Puspita L., & Yulin L. 2014. Population and Diversity of Endophytic Bacteria Associated with Medicinal Plant *Curcuma zedoaria*. *Microbiology Indonesia*, 8(2):65–72.

Tiwari, P., & Bae, H. (2020). Horizontal Gene Transfer and Endophytes: An Implication for the Acquisition of Novel Traits. *Plants (Basel, Switzerland)*, 9(3):305. https://doi.org/10.3390/plants9030305.

Tshikhudo, P. P., Ntushelo, K., & Mudau, F. N. (2023). Sustainable Applications of Endophytic Bacteria and Their Physiological/Biochemical Roles on Medicinal and Herbal Plants: Review. *Microorganisms*, 11(2):453. https://doi.org/10.3390/microorganisms11020453.

Utami P., Puspaningtyas D. E., & Indah Y. 2013. *The miracle of herbs*. Jakarta: Agro Media Pustaka.

Wen, J., Okyere, S. K., Wang, S., Wang, J., Xie, L., Ran, Y., & Hu, Y. (2022). Endophytic Fungi: An Effective Alternative Source of Plant-Derived Bioactive Compounds for Pharmacological Studies. *Journal of Fungi*, 8(2):205. https://doi.org/10.3390/jof8020205.

Widowati Tiwit, D. 2016. Isolasi dan Identifikasi Kapang Endofit dari Tanaman Kunyit sebagai Penghasil Antioksidan. *Biopropal Industri*. 7(1):9–16.

Yan, Y., Xia, X., Fatima, A., Zhang, L., Yuan, G., Lian, F., & Wang, Y. (2024). Antibacterial Activity and Mechanisms of Plant Flavonoids Against Gram-Negative Bacteria Based on the Antibacterial Statistical Model. *Pharmaceuticals*, 17(3):292–292. https://doi.org/10.3390/ph17030292.