

Biosynthetic potential of endophytic fungi associated with breadfruit (*Artocarpus communis* forst.) for antimicrobial agent production

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ABSTRACT: The rising incidence of antibiotic resistance in pathogenic bacteria necessitates the exploration of endophytic fungi as alternative sources of novel antimicrobial compounds. This study aimed to isolate, characterize, and evaluate the antimicrobial potential of endophytic fungi derived from the leaves and stems of breadfruit (*Artocarpus communis* Forst) against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Isolation was conducted using surface sterilization and direct plating methods on Potato Dextrose Agar (PDA). Fungal isolates were characterized macroscopically and microscopically. Antimicrobial potential was assessed in two stages: preliminary screening using the dual culture antagonism method, followed by confirmation testing of secondary metabolites produced via liquid fermentation in Potato Dextrose Broth (PDB) using the agar well diffusion method. Eight pure isolates were successfully obtained: three from leaves and five from stems. Preliminary screening indicated that stem-derived isolates exhibited superior antimicrobial activity compared to leaf-derived isolates. Secondary metabolite screening revealed that isolate B1a-Ir effectively inhibited *S. aureus* with an inhibition zone of 9.19 ± 0.17 mm, surpassing the positive control (chloramphenicol). Furthermore, isolates B2a-Ir and B3b-Ir exhibited strong antifungal activity against *C. albicans* with inhibition zones of 13.09 ± 0.49 mm and 13.93 ± 0.17 mm, respectively, surpassing the positive control (ketoconazole). This study highlights that endophytic fungi from breadfruit stems are promising sources of bioactive compounds for future antimicrobial therapy development, particularly against *S. aureus* and *C. albicans*.

KEYWORDS: Antimicrobial activity; *Artocarpus communis* forst.; endophytic fungi; liquid fermentation; secondary metabolites.

INTRODUCTION

Antimicrobial resistance (AMR) is a serious global public health problem that has substantially reduced the effectiveness of conventional antibiotics and antifungal agents in the treatment of infectious diseases [1]. This phenomenon is driven not only by the irrational use of antibiotics in healthcare facilities but is also exacerbated by the failure of the antibiotic market to provide adequate economic incentives for the pharmaceutical industry to develop new drugs, thereby hindering therapeutic innovation [2]. Consequently, multidrug-resistant pathogens are evolving faster than the human capacity to discover new therapeutic agents [3]. This situation necessitates the exploration of new bioactive compounds from natural sources that possess unique mechanisms of action to overcome resistant pathogenic infections [4],[5].

Endophytic fungi, which colonize the internal tissues of healthy plants without causing disease symptoms, are increasingly recognized as a valuable source of novel antimicrobial compounds because of their exceptional biosynthetic potential [5], [6]. The mutualistic symbiosis between endophytic fungi and their host plants enables these microorganisms to produce secondary metabolites that contribute to plant defense against microbial pathogens [7],[8]. Endophytic fungi frequently produce secondary metabolites with chemical structures similar to, or even more complex than, those of their host plants, reflecting long-term co-evolution and metabolic adaptation [9], [10]. Moreover, the exploitation of endophytic fungi represents a sustainable and environmentally friendly approach to natural product discovery, as bioactive metabolites can be produced through controlled laboratory fermentation without depleting natural plant populations [11].

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Breadfruit (*Artocarpus communis* Forst.) is a medicinal plant widely used in traditional medicine for the treatment of skin infections, abscesses, and wounds [12]. Previous studies have demonstrated that extracts from various tissues of *A. communis* exhibit potent antimicrobial activity against *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [13]. These antimicrobial properties have been attributed to the presence of bioactive secondary metabolites, including flavonoids, saponins, terpenoids, and phenolic compounds, within breadfruit tissues [12]. Given the rich phytochemical profile of *A. communis*, its associated endophytic fungi represent a promising reservoir of microorganisms capable of producing antimicrobial metabolites with potential pharmaceutical applications [5].

Although the potential of endophytes from medicinal plants has been extensively studied, comparative studies regarding the production dynamics of secondary metabolites from endophytic fungi isolated from different plant organs remain limited. Differences in physiological characteristics and micro-environments between breadfruit leaves and stems influence the community of endophytes that colonize them [14]. These ecological niche differences support the hypothesis that fungi from different tissues will yield distinct secondary metabolite profiles and production kinetics in inhibiting pathogens [15]. Systematic exploration based on plant tissue type is crucial for maximizing the yield of bioactive compounds [16].

This research aims to isolate and screen endophytic fungi from the leaf and stem tissues of breadfruit (*Artocarpus communis* Forst.) originating from the Sewan Neglasari area, Tangerang, and to evaluate their antimicrobial potential. Furthermore, this study maps the production dynamics of secondary metabolites from superior endophytic fungi during liquid fermentation to determine the optimal time crucial for maximizing the yield of active compounds [16]. The activity evaluation was conducted against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* to provide a comprehensive overview of the potential of breadfruit endophytic fungi as a source of new antimicrobial agents effective in overcoming the problem of microbial resistance [17].

▪ MATERIALS AND METHODS

Materials and specimen determination

Sample Collection

The plant materials used in this study consisted of mature leaf tissues and stem twigs of breadfruit (*Artocarpus communis* Forst.), which were collected by random sampling from three healthy trees in the Sewan Neglasari area, Tangerang, Banten, Indonesia. Botanical identification was performed at the Herbarium Bogoriense, National Research and Innovation Agency (BRIN), Cibinong, Bogor, Indonesia. The plant specimen was confirmed as *Artocarpus communis* Forst. based on Determination Letter No. B-933/IV/DI.05.07/4/2022, ensuring the taxonomic validity of the plant material used in this study.

Chemicals and microbial agents

The chemicals used included sterile distilled water, 5.25% Sodium hypochlorite (NaOCl) (Merck, Germany), 70% Ethanol (Merck, Germany), methylene blue (Merck, Germany), and 0.9% NaCl (Merck, Germany). The growth media used included Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), and Nutrient Agar (NA) (Difco, Detroit, USA). The test microbes included the Gram-positive bacterium *Staphylococcus aureus*, the Gram-negative bacterium *Escherichia coli*, and the fungus *Candida albicans*, which were obtained from the laboratory culture collection. The antibiotic reference standards used were 50 µg/mL chloramphenicol and 250 µg/mL ketoconazole as an antifungal.

Sterilization of equipment and materials

All glassware and culture media were sterilized in an autoclave at 121 °C under 1.5 atm pressure for 15 min.

Preparation of Nutrient Agar (NA) media

Approximately 28 g of Nutrient Agar (NA) powder was weighed and dissolved in distilled water to a final volume of 1000 mL in an Erlenmeyer flask. The medium was heated while continuously stirred using a hot plate magnetic stirrer until completely dissolved. The flask was plugged with cotton wool and covered

with aluminum foil. The prepared medium was sterilized by autoclaving at 121 °C and 1.5 atm for 15 min, then allowed to cool and stored at 4 °C until use [18].

Preparation of Potato Dextrose Agar (PDA) medium

Approximately 39 g of Potato Dextrose Agar (PDA) powder was weighed and dissolved in distilled water to a final volume of 1000 mL in an Erlenmeyer flask. The medium was heated while continuously stirred using a hot plate magnetic stirrer until completely dissolved. The flask was plugged with cotton wool and covered with aluminum foil. The prepared medium was sterilized by autoclaving at 121 °C and 1.5 atm for 15 min, then allowed to cool and stored in a refrigerator until use [18].

Preparation of Potato Dextrose Broth (PDB) medium

Approximately 26.4 g of Potato Dextrose Broth (PDB) powder was weighed and dissolved in distilled water to a final volume of 1000 mL in an Erlenmeyer flask. The medium was heated while continuously stirred using a hot plate magnetic stirrer until completely dissolved. The flask was plugged with cotton wool and covered with aluminum foil. The prepared medium was sterilized by autoclaving at 121 °C and 1.5 atm for 15 min, then allowed to cool and stored in a refrigerator until use [10].

Isolation and purification of endophytic fungi

Endophytic fungi isolation was performed using the direct plating method [19]. Breadfruit leaf and twig samples were washed under running water for 5 minutes, then leaves were cut into 1 cm x 1 cm pieces and twigs into 1 cm pieces using a sterile knife [7]. Surface sterilization of the samples was carried out by immersing them in 70% ethanol for 1 minute, 5.25% sodium hypochlorite for 1 minute, and 70% ethanol again for 1 minute, followed by rinsing with sterile distilled water for 3-5 seconds [20]. The sterile sample pieces were placed on sterile filter paper, then planted on Petri dishes containing PDA medium in duplicate (3 leaf pieces per dish). The media were incubated at room temperature for 7-10 days. The final rinse water was inoculated onto PDA medium as a sterilization control [7].

The growing endophytic fungi were purified on a new PDA medium. Isolates with different morphologies were inoculated onto new PDA media and incubated for 7-10 days. The purification process was repeated until pure isolates were obtained [21]. Pure isolates were prepared in duplicate as working cultures and stock cultures, incubated for 7-10 days, and stored in a refrigerator [7].

Characterization of endophytic fungi

Macroscopic characterization included observation of colony color, texture, surface (granular, raised, smooth), zonation, radial lines, and reverse colony color [22]. Microscopic characterization was performed using a staining method. Pure fungal isolates were cut into 1x1 cm pieces, placed on a sterile glass slide, stained with methylene blue, covered with a cover glass, and observed under a microscope [23] to identify the presence of hyphal septa, shape, and branching patterns.

Rejuvenation of test microbes

The bacterial test strains, *Staphylococcus aureus* and *Escherichia coli*, were rejuvenated on Nutrient Agar (NA) slants, whereas the fungal test strain, *Candida albicans*, was rejuvenated on Potato Dextrose Agar (PDA) slants. The bacterial cultures were incubated at 37 °C for 24 h, while the fungal culture was incubated at 30 °C for 48 h to ensure optimal viability [22],[24].

Preparation of test microbial suspension

The microbial suspension was prepared by aseptically transferring a single colony of each rejuvenated microorganism into a sterile tube containing sterile 0.9% NaCl solution and vortexing until homogeneous [25],[26]. The turbidity of the initial suspension was adjusted to match the 3.0 McFarland standard (approximately 9.0×10^8 CFU/mL), which served as the initial stock suspension. The stock suspension was subsequently serially diluted to obtain a working inoculum of approximately 1×10^6 CFU/mL for the antimicrobial assay [27]. Initially, 1 mL of the stock suspension was transferred into a tube containing 9 mL of sterile 0.9% NaCl to obtain approximately 10^8 CFU/mL. Subsequently, 1 mL of the 10^8 CFU/mL suspension was transferred into another tube containing 9 mL of sterile 0.9% NaCl to obtain approximately 10^7 CFU/mL. Finally, 1 mL of the 10^7 CFU/mL suspension was transferred into a final tube containing 9 mL of sterile 0.9%

NaCl to obtain the working inoculum of approximately 1×10^6 CFU/mL, which was used immediately for the antimicrobial assays [22].

Selection of potential endophytic fungi via antagonism test

The selection of endophytic fungi with antimicrobial potential was performed using the antagonism test method (dual culture technique). Approximately 1000 μ L of test microbe suspension (10^6 CFU/mL) was spread on a Petri dish, poured with 15 mL of agar medium (NA for bacteria, PDA for fungi), shaken gently, and allowed to solidify [28]. Pure fungal isolates were cut into 6 mm diameter plugs and placed at the center of the Petri dish already inoculated with the test microbes. The dishes were incubated at 37 °C for 1–2 days. Antimicrobial activity was indicated by the formation of an inhibition zone around the fungal colony [29].

Secondary metabolite production

Secondary metabolite production was carried out using liquid fermentation with PDB medium. Selected pure fungal isolates were cut into 1 $\times$ 1 cm pieces and inoculated into 20 mL of PDB medium in a 100 mL Erlenmeyer flask [29]. Fermentation was performed using a vertical shaker at 150 rpm for 5 days at room temperature. Culture samples were taken every 6 hours and placed into sterile Eppendorf tubes. The supernatant was separated from the biomass by centrifugation at 3000 rpm for 20 minutes, then stored in a refrigerator for activity testing [22].

Antimicrobial assay

The antimicrobial activity of the fermentation supernatant was determined using the agar well diffusion method combined with the pour plate technique. Approximately 1000 μ L of the standardized test microbe suspension (10^6 CFU/mL) was inoculated into a Petri dish, followed by 15 mL of molten agar medium (NA/PDA), mixed thoroughly, and allowed to solidify [27]. Once solidified, wells with a diameter of 6 mm were created in the agar using a sterile cork borer [30]. Approximately 50 μ L of the fermentation supernatant was introduced into each well. Chloramphenicol solution (50 μ g/mL) was used as the positive control for bacteria, and ketoconazole solution (250 μ g/mL) for fungi, while sterile distilled water served as the negative control. The plates were incubated at 37 °C for 24 hours. Antimicrobial activity was quantified by measuring the total diameter of the inhibition zone using a vernier caliper [31], [38]. The net inhibition zone was calculated by subtracting the 6-mm well diameter from the total inhibition zone diameter. All assays were conducted in triplicate to ensure reproducibility.

RESULTS

Botanical and ecological verification of the host

The botanical identity of the breadfruit plant (*Artocarpus communis* Forst) was confirmed at the Herbarium Bogoriense, National Research and Innovation Agency (BRIN), Indonesia (Specimen ID: B-933/IV/DI.05.07/4/2022). To ensure maximum metabolic activity and secondary metabolite concentration, sampling of mature leaves and stems was conducted during the morning hours.

Isolation and morphological characterization of endophytic fungi

The isolation procedure yielded eight distinct pure endophytic fungal isolates, comprising three isolates from leaf tissues (D1a-Ir, D1b-Ir, D2a-Ir) and five isolates from stem tissues (B1a-Ir, B1b-Ir, B2a-Ir, B3a-Ir, B3b-Ir). Macroscopic analysis of the colonies grown on agar medium revealed significant phenotypic diversity. Colony morphologies ranged from granular to velvety and cottony textures, with surface colors displaying a broad spectrum, including white, yellow, orange, whitish-green, and black (Figure 1).

Microscopic observations (Figure 2) confirmed the structural diversity among the isolates. The majority of the isolates exhibited non-septate hyphae, with the notable exception of B2a-Ir, which showed septate hyphae. Furthermore, distinct reproductive structures were observed: isolates D1b-Ir, B1a-Ir, and B1b-Ir produced conidia and conidiophores. Conversely, isolates D2a-Ir, B2a-Ir, and B3b-Ir possessed sporangiophores and spores of varying morphologies, including oval and rounded forms. Isolate B3a-Ir was unique in exhibiting non-branching hyphae. These profound morphological differences strongly indicate the presence of distinct fungal groups colonizing the host plant tissues.

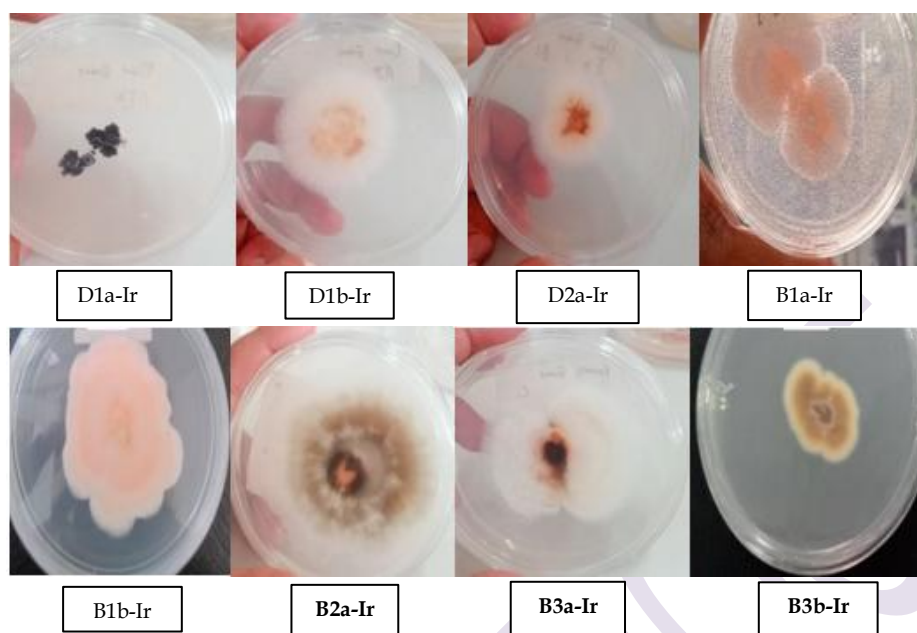


Figure 1: Macroscopic characteristics of isolates D1a-Ir to B3b-Ir

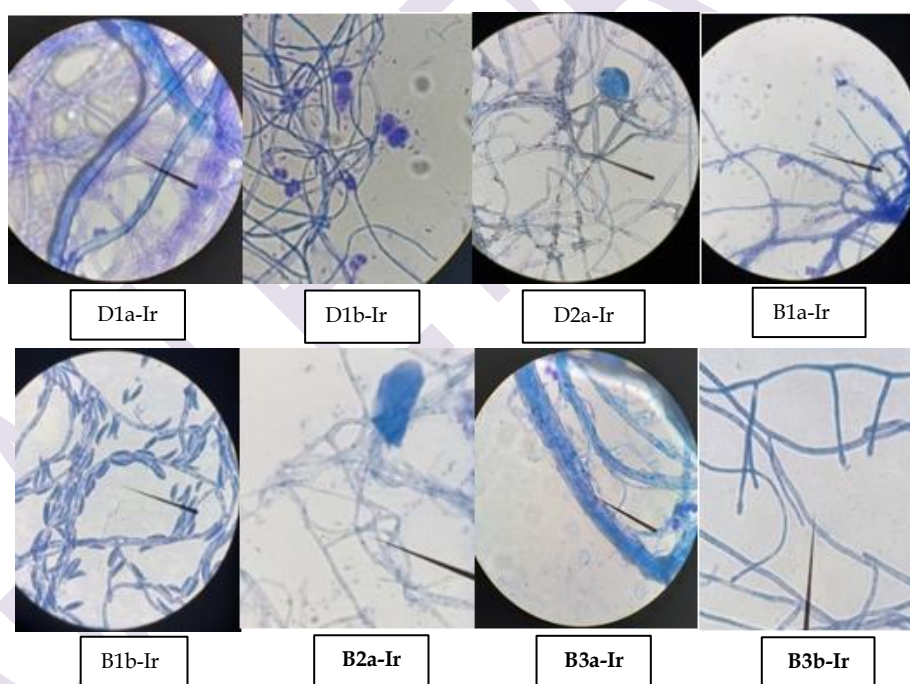


Figure 2. Microscopic structures of endophytic fungal isolates D1a-Ir to B3b-Ir at ×1000 magnification.

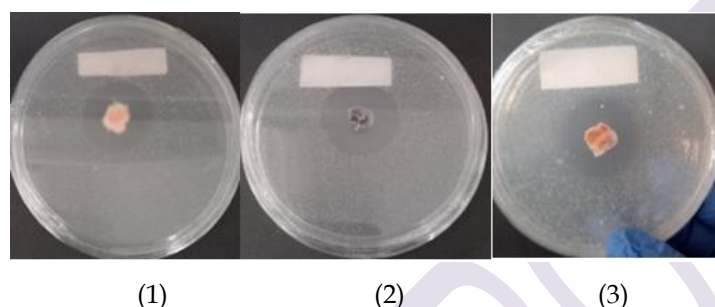
Selection of potential endophytic fungi through antagonism assays

Preliminary screening via the dual culture technique demonstrated that only three isolates derived from stem tissues, B1a-Ir, B2a-Ir, and B3b-Ir, exhibited significant antagonistic activity against the tested pathogens (Table 1). Isolate B2a-Ir showed the highest activity against *Candida albicans* (18.85 mm), while B1a-Ir demonstrated the strongest inhibition against *Staphylococcus aureus* (15.15 mm). Notably, none of the isolates showed inhibitory activity against the Gram-negative bacterium *Escherichia coli*. These three isolates were prioritized for further fermentation analysis based on the inhibition zones shown in Figure 3.

Table 1. Inhibition zones of endophytic fungal isolates in the preliminary antagonism test.

Isolate Code	Inhibition zone diameter (mm)		
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
D1a-Ir	-	-	-
D1b-Ir	-	-	-
D2a-Ir	-	-	-
B1a-Ir	15.15	-	-
B1b-Ir	-	-	-
B2a-Ir	-	-	18.85
B3a-Ir	-	-	-
B3b-Ir	-	-	16.45

Note: - indicates that no inhibition zone was observed.

**Figure 3.** Documentation of inhibition zones from the antagonism test on potential isolates.

1. Isolate B1a-Ir against *Staphylococcus aureus*
2. Isolate B2a-Ir against *Candida albicans*
3. Isolate B3b-Ir against *Candida albicans*

Analysis of secondary metabolite products from endophytic fungi

Liquid fermentation of the selected isolates (B1a-Ir, B2a-Ir, and B3b-Ir) in Potato Dextrose Broth (PDB) demonstrated that the production of antimicrobial secondary metabolites was time-dependent, reaching its peak during the stationary growth phase (idiophase), as shown in Table 2. The fermentation supernatants exhibited significant antimicrobial activity, with some isolates producing inhibitory effects comparable to or greater than those of the positive controls. Specifically, isolate B1a-Ir exhibited strong antibacterial activity against *Staphylococcus aureus*, with a net inhibition zone of 9.19 ± 0.17 mm, whereas isolates B2a-Ir and B3b-Ir displayed potent antifungal activity against *Candida albicans*, with net inhibition zones of 13.09 ± 0.49 mm and 13.93 ± 0.17 mm, respectively, exceeding that of the positive control (ketoconazole). The antimicrobial activities of the fermentation supernatants are illustrated in Figure 4 by the presence of distinct inhibition zones surrounding the wells. Notably, maximum metabolite production was achieved within 72–84 h of fermentation, indicating efficient biosynthesis of antimicrobial metabolites under the applied culture conditions.

Table 2. Highest inhibition zones of endophytic fungal supernatants from PDB cultures.

Antimicrobial activity test	Endophyte isolates	Highest inhibition zone (mm $\pm$ SD)	Time (h)
Against <i>Staphylococcus aureus</i>	B1a-Ir	9.19 ± 0.17	84
	Positive Control	3.18 ± 0.17	NA
	Negative Control	0.00 ± 0.00	NA
Against <i>Candida albicans</i>	B2a-Ir	13.09 ± 0.49	78
	B3b-Ir	13.93 ± 0.17	72
	Positive Control	11.65 ± 0.36	NA
	Negative Control	0.00 ± 0.00	NA

Note: Values represent net inhibition zones, calculated by subtracting the 6-mm well diameter from the total inhibition zone diameter. NA indicates that the fermentation time was not applicable for the positive and negative controls.

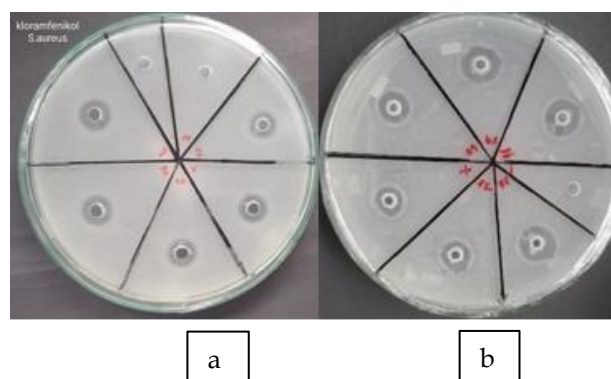


Figure 4. Antimicrobial activity of endophytic fungi supernatants from *Artocarpus communis* Forst. against pathogenic microbes.

(a) Inhibition zone of isolate B1a-Ir against *Staphylococcus aureus*;

(b) Inhibition zone of isolate B3b-Ir against *Candida albicans*.

Note: (+) Positive control: chloramphenicol solution (50 µg/mL) for antibacterial assays and ketoconazole solution (250 µg/mL) for antifungal assays; (-) Negative control: sterile distilled water. The presence of a clear inhibition zone surrounding the well indicates antimicrobial activity.

Kinetic analysis (Figures 5.1–5.3) provided a detailed profile of antimicrobial activity throughout the fermentation period. Figure 5.1 illustrates the antibacterial activity of isolate B1a-Ir against *Staphylococcus aureus*, showing a gradual increase in the net inhibition zone, which reached a maximum of 9.19 ± 0.17 mm after 84 h of fermentation. Similarly, Figures 5.2 and 5.3 demonstrate the time-dependent antifungal activity of isolates B2a-Ir and B3b-Ir against *Candida albicans*, with maximum net inhibition zones of 13.09 ± 0.49 mm and 13.93 ± 0.17 mm observed after 78 h and 72 h of fermentation, respectively. The subsequent decline in antimicrobial activity observed in all three isolates may be attributed to the degradation of bioactive metabolites, nutrient depletion, or the accumulation of inhibitory metabolic by-products, which collectively reduced the production of antimicrobial secondary metabolites.

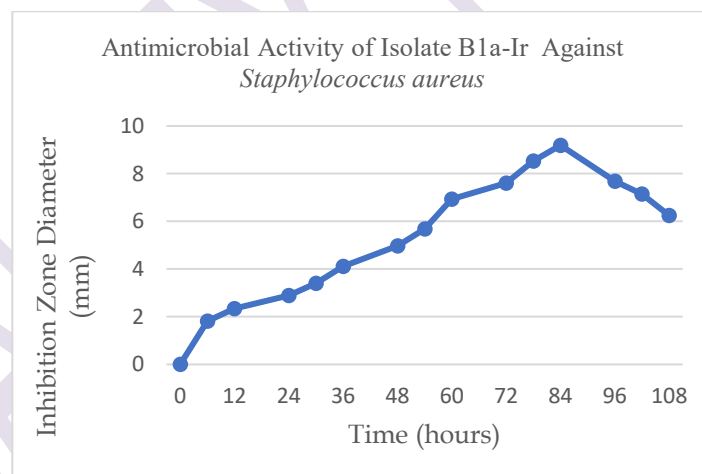


Figure 5.1 Inhibition zone activity curve of isolate B1a-Ir against *Staphylococcus aureus*.

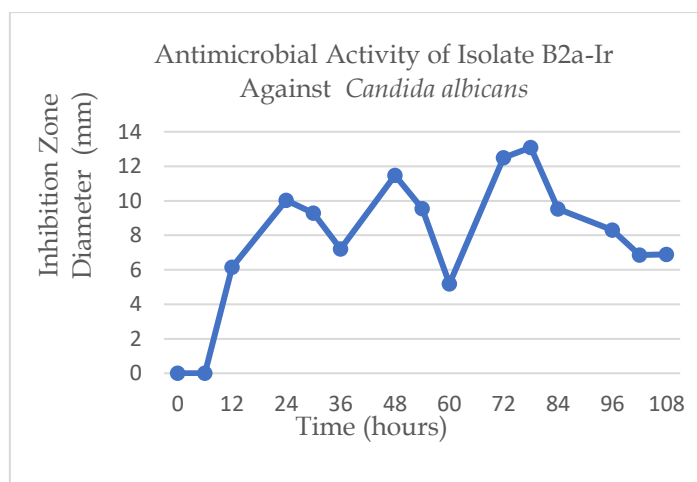


Figure 5.2 Inhibition zone activity curve of isolate B2a-Ir against *Candida albicans*.

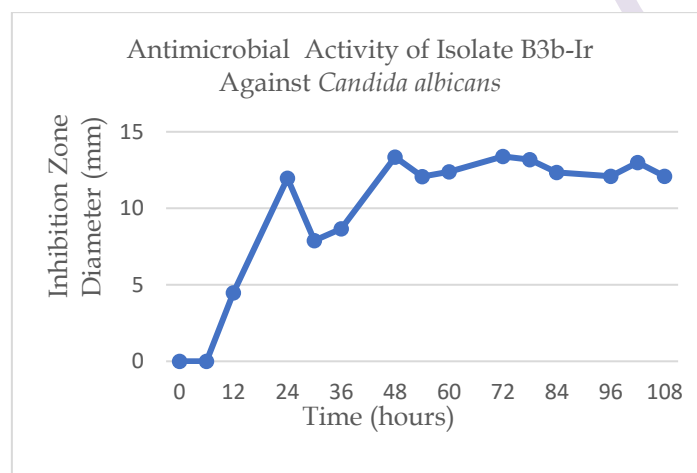


Figure 5.3 Inhibition zone activity curve of isolate B3b-Ir against *Candida albicans*.

DISCUSSION

Botanical and ecological verification of the host

The precise identification of the host plant taxonomy is a crucial foundation in microbial bioprospecting, as taxonomic misidentification can lead to erroneous conclusions regarding the host-microbe relationship. The determination of *Artocarpus communis* at the Herbarium Bogoriense ensured that the generated data derived from a botanically valid species, considering that the endophytic fungal community is profoundly influenced by host phylogeny and biogeographic factors [14]. Sampling was conducted in the Sewan Neglasari area, Tangerang, Banten, topographically located in the lowlands at an altitude of less than 10 meters above sea level. This region possesses a tropical monsoonal climate with high atmospheric humidity and an average annual rainfall ranging between 1,500–2,500 mm. Such warm and humid environmental conditions strongly support rapid vegetation growth while simultaneously triggering high endophytic microbial diversity, as a high-humidity environment facilitates fungal spores in adhering to and infecting host tissues [34], [47]. Moreover, this environment promotes a high rate of horizontal gene transfer between the environment and the plant, further diversifying the endophytic population and enhancing their adaptive biosynthetic capabilities [47].

Sampling focused on mature organs (leaves and stems) in the morning. This procedure is based on an ecophysiological strategy; mature tissues provide a longer colonization window for fungi to adapt to the host's secondary chemistry, allowing for a more stable and established symbiotic relationship [47]. Furthermore, in the morning, the plant metabolism is at its optimal point, supporting the accumulation of long-term defense metabolites unique to that endophyte community structure. This phenomenon is related to the high

translocation of photosynthates on the previous night, triggering a more intensive synthesis of active compounds in the morning [49]. Therefore, morning sampling ensures that the captured endophytes are actively involved in the host's metabolic defense pathways, increasing the likelihood of isolating potent bioactive producers [13].

Isolation and morphological characterization of endophytic fungi

The successful isolation of eight pure isolates demonstrates the effectiveness of surface sterilization using ethanol and NaOCl, ensuring that the fungi obtained are true endophytes rather than epiphytic contaminants adhering to the surface of the plant organs [33]. The use of direct plating methods allowed for the isolation of slow-growing endophytes that might otherwise be overlooked by other methods, ensuring a comprehensive representation of the fungal community [34]. The striking phenotypic diversity observed in the macroscopic characteristics suggests that breadfruit tissues host a heterogeneous community of fungi. The variation in colors and textures corresponds with findings from similar studies indicating that different environmental conditions and host tissues influence the morphology of endophytic fungi [32]. This diversity is not merely superficial; it suggests distinct metabolic pathways adapted to the nutrient composition of different plant parts, increasing the chances of discovering structurally diverse compounds [47]. Microscopic observations provided deeper insight into the structural diversity of the isolates. Variations in reproductive structures, such as conidia shapes, sporangioophores, and hyphal branching patterns, allowed for distinct differentiation between the isolates (e.g., B2a-Ir and B1a-Ir). These morphological variations are sufficient to classify the isolates into distinct phenotypic groups, which is the primary requirement for screening diverse biosynthetic capacities within the host tissues [4]. This morphological diversity indicates that the breadfruit tree acts as a natural therapeutic "reservoir" for various fungal taxa with diverse abilities to produce bioactive compounds. Furthermore, the prevalence of distinct reproductive structures suggests that these fungi have evolved specialized mechanisms for propagation within the hostile environment of the plant tissue, which is often linked to the production of specialized secondary metabolites [35].

Selection of potential endophytic fungi

Screening results demonstrated the superiority of endophytic fungi derived from the breadfruit stem in inhibiting the growth of pathogenic microbes. Isolate B1a-Ir was specifically active against *Staphylococcus aureus*, while isolates B2a-Ir and B3b-Ir exhibited high inhibitory power against *Candida albicans*. The activity of these stem isolates is closely related to the microenvironment of the stem tissue, which is rich in phenolic and terpenoid compounds serving as the natural defense mechanism of the wood; consequently, colonizing endophytes tend to assimilate the biosynthetic capabilities for similar defense compounds to inhibit pathogens [36],[48]. This assimilation is likely facilitated by horizontal gene transfer, allowing the fungi to produce compounds that are chemically similar to the host's own defense mechanisms, thus ensuring compatibility while providing added protection [8].

On the other hand, the inactivity of the isolates against the Gram-negative bacterium *Escherichia coli* is due to the bacteria's intrinsic resistance. The cell wall structure of *E. coli* possesses an outer membrane of lipopolysaccharide (LPS) that acts as a remarkably robust mechanical and chemical barrier. This LPS component limits membrane permeability to many antimicrobial compounds [37]. Furthermore, *E. coli* possesses a highly efficient efflux pump system that expels foreign compounds, including antibiotics or secondary metabolites that enter the cell, before they reach their molecular targets [24], [29]. This resistance underscores the need for compounds with novel mechanisms of action, perhaps targeting the LPS synthesis itself, which these endophytes might produce [39].

The ability of isolates B2a-Ir and B3b-Ir to inhibit the fungus *C. albicans* is likely supported by the production of secondary metabolites capable of disrupting cell membrane integrity through sterol disruption mechanisms or inhibition of cell wall synthesis [24], [29]. The similarity of the cell membrane composition between the endophytes and the pathogen necessitates a precise mechanism of action that only affects the pathogen, hinting at a highly evolved, specific biosynthetic pathway [41]. The superiority of breadfruit stem endophytic fungi in producing these antimicrobial compounds highlights the immense potential of its host as a reservoir of microbes that produce novel active compounds.

Fermentation dynamics and metabolite production

Fermentation dynamics in PDB medium indicated that secondary metabolite production is time-dependent. The peak activity between 72 and 78 hours confirms that bioactive compounds are synthesized during the stationary phase or idiophase. In this phase, nutrient limitation triggers microbes to produce secondary metabolites as a survival mechanism or cell-to-cell communication tool (quorum sensing) [42]. The timing of this peak indicates that the compounds are not mere byproducts of rapid growth but are complex molecules developed for defense or competition, typical of idiophase metabolism [43].

The decline in activity after 84 hours is most likely caused by the degradation of active compounds by the fungus's own extracellular enzymes or pH fluctuations that disrupt the stability of the metabolite's chemical structure [44]. This fluctuation is closely related to the OSMAC (One Strain Many Compounds) principle, wherein the metabolite profile can change drastically based on fermentation duration, oxygen supply, and incubation conditions [45]. Therefore, identifying the exact moment of maximum metabolite accumulation is crucial for efficient extraction and purification, maximizing yield in bioprocessing [41].

The test results indicating that the activity of isolates B2a-Ir and B3b-Ir falls within the "strong" category are highly promising findings. The strength of this "strong" activity indicates the presence of aggressive compounds, most likely from the terpenoid or alkaloid group, capable of disrupting the integrity of the fungal cell membrane through sterol disruption mechanisms or inhibition of cell wall synthesis [40]. The concentration of activity in the stem isolates also strengthens the theory of Horizontal Gene Transfer (HGT) or co-evolution between the host and its endophytes. Considering that breadfruit stems are rich in phenolic compounds, flavonoids, and terpenoids that serve natural defense functions [46], the endophytic fungi have likely assimilated similar biosynthetic pathways or act as an "additional phenotype" that reinforces the host's defense system against pathogens [36].

Regarding the bioprospecting aspect, the morphological diversity and bioactivity of the endophytic isolates from *Artocarpus communis* affirm their potential as a source of novel chemical scaffolds that remain largely underexplored [6]. The integration between plant metabolism and its symbionts creates an efficient biosynthetic system for producing natural products with high medical value. The stable plant habitat within the stem tissue provides an ecological niche that supports the growth of endophytes with more complex biosynthetic profiles compared to those in leaf tissue [32]. This stability allows for the long-term co-evolution necessary for the development of sophisticated chemical defenses, making stem endophytes particularly valuable for drug discovery [35]. Further exploitation of these potential isolates, particularly through molecular identification (ITS rDNA sequencing), genomic approaches, and medium optimization using epigenetic modifier triggers, constitutes a strategic step in the endeavor to discover new antimicrobial agents to overcome the global resistance crisis [5].

CONCLUSION

Eight morphologically distinct endophytic fungal isolates were successfully recovered from breadfruit (*Artocarpus communis* Forst.) tissues, demonstrating considerable macroscopic and microscopic diversity. Among these, three stem-derived isolates (B1a-Ir, B2a-Ir, and B3b-Ir) exhibited antimicrobial activity in the antagonism assay. Isolates B2a-Ir and B3b-Ir showed potent antifungal activity against *Candida albicans*, whereas isolate B1a-Ir exhibited antibacterial activity against *Staphylococcus aureus*. None of the isolates inhibited the growth of *Escherichia coli*. Liquid fermentation in Potato Dextrose Broth (PDB) indicated that the highest production of antimicrobial secondary metabolites occurred during the stationary phase (idiophase), with peak bioactivity observed between 72 and 84 h of fermentation at an agitation speed of 150 rpm. The fermentation supernatants of isolates B2a-Ir and B3b-Ir exhibited greater inhibitory activity against *C. albicans* than the positive control (ketoconazole), highlighting the potential of breadfruit-derived endophytic fungi as promising sources of antimicrobial compounds. These findings support further bioprospecting and chemical characterization of endophytic fungi associated with *A. communis* for the discovery of novel antimicrobial agents.

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