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Biocontrol Potential and Mechanisms of Cocoa-Fermentation Yeasts Against Fungal Pathogens of Cocoa Pods

Dalia Sukmawati [1,2*], Shabrina Nida Al Husna [3], Allika Firhandini [1], Dwi Ningsih Susilowati [4], R. Haryo Bimo Setiarto [5], Lutfi Anshory [5], Lutfi Anggadhania [5], Mavis Agyeiwaa Acheampong [6], Hesham Ali El Enshasy [7]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jakarta 13220, Indonesia; Dalia-Sukmawati@unj.ac.id (D.S.); allikaf19@gmail.com (A.F.);
[2] Universitas Negeri Culture Collection, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Jakarta 13220, Indonesia; Dalia-Sukmawati@unj.ac.id;
[3] Department of Microbiology, Institut Teknologi Bandung, Bandung 40132, Indonesia; shabrina.nida@gmail.com;
[4] Research Centre for Horticulture, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor km 46, Cibinong Bogor 16915, West Java Indonesia; d_nengsusi@yahoo.com;
[5] Research Center for Applied Microbiology, National Research and Innovation Agency, KST Soekarno, West Java 16911, Indonesia; rhar002@brin.go.id (R.H.B.S); lutf012@brin.go.id (L.A); lutf009@brin.go.id (L.A.);
[6] Department of Crop Science, University of Ghana, P. O. Box LG 44, Legon, Accra, Ghana; maacheampong@ug.edu.gh;
[7] Innovation Centre in Agritechnology in Advanced Bioprocessing (ICA), Universiti Teknologi Malaysia, Higher Education Hup, Pagoh, Johor, Malaysia; henshasy@ibd.utm.my;
* Correspondence: Dalia-Sukmawati@unj.ac.id;

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Abstract: This study investigated the inhibitory potential and mechanisms of four yeast isolates obtained from cocoa bean fermentation against three fungal pathogens of cocoa pods. Molecular identification revealed that the yeast isolates UNJCC Y-158 and UNJCC Y-159 corresponded to *Rhodotorula alborubescens* and *Meyerozyma guilliermondii*, respectively, while UNJCC Y-160 and UNJCC Y-161 were *Pichia kudriavzevii*. All four yeast isolates demonstrated inhibitory potential against the tested fungi pathogens of cocoa pods: *Fusarium decemcellulare* UNJCC F9, *Fusarium solani* UNJCC F18, and *Colletotrichum siamense* UNJCC F14. The results showed that *Pichia kudriavzevii* UNJCC Y-160 and UNJCC Y-161 exhibited the highest inhibition against the tested fungi, with inhibition percentages ranging from 12.61–34.64%. In contrast, *Rhodotorula alborubescens* UNJCC Y-158 and *Meyerozyma guilliermondii* UNJCC Y-159 displayed weaker inhibition, with inhibition percentages ranging from 2.93–10.68%. Antagonism, hyperparasitism, and the production of volatile compounds were observed as mechanisms for inhibition. Microscopic observations revealed that yeast cells attach to the fungal hyphae, causing morphological changes such as thinning and shrinking, likely due to the secretion of hydrolytic enzymes, including cellulase, produced by the yeasts. This study offers a promising alternative to chemical fungicides by utilising natural yeast isolates as biocontrol agents, thereby contributing to sustainable and environmentally friendly cocoa disease management strategies.

Keywords: biocontrol activity; antagonistic activity; yeast; fungal pathogens of cocoa; cocoa pods; volatile compounds.

1. Introduction

Cocoa (*Theobroma cacao* L.) is a major export commodity that plays a crucial role in supporting regional economies in several provinces of Indonesia, including Aceh, South Sulawesi, Southeast Sulawesi, Central Sulawesi, West Sulawesi, and North Sumatra [1]. However, global cocoa production has been facing a decline, with a reported 14% reduction during the 2023/2024 cocoa season [2]. Indonesia is experiencing the most significant decline in cocoa production in the Asian region [3]. One of the primary factors contributing to this decline is the deterioration of cocoa bean quality and quantity due to various pod diseases, particularly those caused by fungal pathogens [4,5]. Several fungal species have been identified as the main causative agents of cocoa pod rot, including *Phytophthora palmivora*, *Moniliophthora roreri*, *Fusarium* spp., *Aspergillus niger*, *Gloeosporium* spp., *Fusarium decemcellulare*, *Fusarium solani*, and *Colletotrichum siamense* [6-8].

To date, efforts to control cocoa pod rot have relied on mechanical, physical, and chemical methods, each of which presents challenges and limitations. Mechanical and physical control measures involve pruning infected branches and removing diseased pods to prevent the spread of further fungi. However, these methods may inadvertently facilitate the dispersal of fungi spores to new areas, even when infected materials are burned [9]. Meanwhile, chemical control is commonly carried out using contact fungicides containing 0.3% copper-based active ingredients. Although these fungicides can be effective in suppressing fungal growth, their prolonged use poses significant environmental and health risks. The accumulation of copper residues in the soil can disrupt the sustainability of agroecosystems, and long-term exposure to fungicides may have adverse effects on human health [10,11]. Given these limitations, there is a growing need for alternative, eco-friendly approaches to managing fungal diseases in cocoa cultivation. One promising approach is the use of microbial biocontrol agents, such as yeast.

Yeast has emerged as a potential biocontrol agent due to its antagonistic properties against fungal pathogens. Unlike pathogenic fungi, yeast does not produce mycotoxins, and unlike bacteria, it does not produce antibiotics, making it a safer and more sustainable alternative for disease control [12-14]. Additionally, yeast is a common epiphytic microorganism that can persist longer on fruit surfaces compared to bacteria, further enhancing its effectiveness as a biocontrol agent [15].

Several mechanisms contribute to yeast's antagonistic activity. One key mechanism is the production of hydrolytic enzymes capable of degrading cell walls, thereby inhibiting fungi's growth and spread [16]. Additionally, yeast can suppress fungal pathogens through competition for nutrients, effectively limiting the resources available for their colonisation. Another mechanism involves the production of killer toxins, which have biocontrol properties and can directly inhibit fungal growth [17].

Yeast have been successfully isolated from various natural substrates, demonstrating their adaptability and potential as biocontrol agents. For instance, studies have reported the isolation of yeast from fermented beverages [18], Red Rose (*Rhododendron* sp.) tree flowers [19], and mulberry (*Broussonetia papyrifera*) fruit [20]. Notably, yeast has also been isolated from cocoa bean fermentation, highlighting its potential role in the cocoa industry [21,22].

Previous research has demonstrated the antagonistic activity of yeast against various fungal pathogens. Pesce [23] reported that yeast isolated from grape fermentation exhibited strong antagonistic properties against *Colletotrichum* spp., responsible for olive diseases. Similarly, Sukmawati [24] found that phylloplane yeast isolated from Bintaro fruit exhibited

inhibitory effects against fungal contaminants in poultry feed. These findings underscore the potential of yeast as a natural and effective biocontrol agent against fungal pathogens.

The Universitas Negeri Jakarta Culture Collection (UNJCC) has a collection of yeast strains isolated from fermented cocoa beans. However, the potential of these yeast strains as biocontrol agents against cocoa fungal pathogens has not yet been explored. Given the increasing interest in sustainable and environmentally friendly disease management strategies, investigating the biocontrol activity of yeast derived from cocoa fermentation could provide valuable insights into alternative control methods for cocoa pod rot.

A recent study by Limtong *et al.* [25] reported that *Torulaspora indica* and *Wickerhamomyces anomalus* exhibited biocontrol potential against *Curvularia lunata* and *Helminthosporium oryzae*, two fungal pathogens responsible for rice seedling rot. These findings suggest that yeast species isolated from plant-based fermentation processes may possess significant biocontrol properties.

Therefore, this study aims to investigate the inhibitory mechanisms of yeast isolates obtained from cocoa bean fermentation on the growth of fungal pathogens that cause cocoa pod disease. By understanding the biocontrol potential of these yeast strains, this research seeks to contribute to the development of sustainable and environmentally friendly disease management strategies for the cocoa industry.

2. Materials and Methods

2.1. Fruit sampling.

Fresh cocoa pods were collected from a cocoa plantation in Sentul, Bogor. Only undamaged pods were selected based on their uniform size and shape. Before use, the cocoa pods underwent surface sterilisation. The sterilisation process involved washing the pods with sterile distilled water, immersing them in a 0.5% sodium hypochlorite (NaOCl) solution for 1 minute, followed by immersion in a 70% ethanol solution for an additional minute, and then rinsing them thoroughly with sterile distilled water.

2.2. Yeast preparation.

Four yeast cells isolated from fermented cocoa beans were obtained from the Universitas Negeri Jakarta Culture Collection (UNJCC). These yeast cultures were used for the antagonistic and biocontrol assays against fungal pathogens isolated from rotten cocoa pods.

2.3. Isolation of fungal pathogens from rotten cocoa pods.

The fungal pathogens used in this study were isolated from rotten cocoa pods and confirmed through pathogenicity tests. The isolation was carried out using a modified direct planting and imprinting method, as described by Bajpai *et al.* [26]. In the direct planting method, small sections (approximately 0.5 cm) were excised from the infected areas at both the tip and the base of the rotten cocoa pod. Meanwhile, in the imprinting method, a 0.5 cm section of Potato Dextrose Agar (PDA) was placed in direct contact with the infected pod surface to facilitate the transfer of fungi. The excised cocoa pod segments and PDA agar samples were then transferred onto PDA plates for fungal growth. Each Petri dish contained two pieces of infected pod tissue or agar medium and was incubated at 29°C for 24 hours. The fungal isolates that emerged were purified using the hyphal tip method to obtain single-colony

cultures, which were subsequently transferred onto PDA slants for stock preservation. The fungal isolates were cultivated on Potato Dextrose Agar (PDA) and incubated at 29°C for seven days before further experimentation.

2.4. Pathogenicity of fungal pathogens.

The pathogenicity test was conducted using a modified Koch's Postulate. The fungi isolated from cocoa fruit were inoculated into healthy cocoa fruit to determine whether the isolate obtained caused rot in cocoa fruit. Before inoculation, the cocoa pods were surface-sterilised. The sterilisation process involved washing the pods with sterile distilled water, followed by immersion in a 0.5% NaOCl solution for one minute, then soaking in 70% ethanol for one minute, and finally rinsing thoroughly with sterile distilled water to remove any remaining disinfectant.

Two uniform wounds with a diameter of 5 mm and a depth of 3 mm were made on the cocoa fruit surface. The wound was inoculated with a fungal suspension. The injured cocoa fruit was placed in a plastic tube and covered with clear plastic. The four corners of the tube were placed with wet cotton to maintain humidity, then incubated for 7 days at a temperature of 28°C. Observations were made every day until the 7th day of incubation after inoculation, by determining the scoring on a scale of 0, 2, 4, 6, and 8 [27], where: 0 = no damage; 2 = ≤ ¼ of the damaged fruit; 4 = ¼ ~ ½ of the damaged fruit; 6 = ½ ~ ¾ of the damaged fruit; 8 = all parts of the fruit are damaged. Identification of pathogenicity was carried out by calculating the percentage of disease incidence (DI) and disease severity (DS) based on Agrios [28] with the formula below:

$$Disease\ Incidence = \frac{n}{N}\ x\ 100\% \qquad (1)$$

Where n is the number of infected plants or plant parts and N is the total number of plants or plant parts observed.

$$Disease\ Severity = \frac{\sum(n \times v)}{Z\ x\ N}\ x\ 100\% \qquad (2)$$

Where n is the number of infected tissues in each severity category, v is the severity score of the infection, Z is the maximum possible severity score, and N is the total number of plants or plant parts observed.

2.5. Molecular identification of yeast and fungal isolates.

The yeast isolates were identified at the species level using molecular methods. DNA was extracted using the boiling method from 48-hour-old cultures grown on Yeast Malt Agar (YMA). PCR amplification targeted the D1/D2 domain of the 26S rRNA gene using universal primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG-3') [29]. The reaction mixture included 100 ng of DNA template and was run under the following conditions: initial denaturation at 95°C for 5 min; 40 cycles. Sequences were analyzed using the BLAST tool (https://blast.ncbi.nlm.nih.gov) for taxonomic identification. Phylogenetic trees were constructed using MEGA 10.0 software with the Neighbor-Joining (NJ) method and 1,000 bootstrap replications.

The pathogenic fungal isolates were also identified using molecular methods. DNA was extracted from 7-day-old cultures grown on Potato Dextrose Agar (PDA). The internal transcribed spacer (ITS) region of rDNA was amplified using the universal fungal primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [30]. PCR reactions were conducted in a 25 µL mixture containing 12.5 µL of GoTaq Green Master Mix, 0.5 µL of each primer, 0.5 µL of DNA template, and 9 µL of nuclease-free water. PCR products were purified and sequenced by PT Genetika Science, Indonesia. Sequences were aligned using BLAST against the NCBI GenBank database for species-level identification. Phylogenetic analysis was performed using MEGA 10.0 software, employing the Neighbor-Joining (NJ) algorithm with 1,000 bootstrap replications.

Finally, all the PCR products were sent to PT Genetika Science in Indonesia for sequencing analysis. The obtained sequences were compared to the GenBank database (www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool Algorithm (BLAST). Phylogenetic trees were constructed using MEGA 10.0 software with the neighbour-joining (NJ) method [30].

2.6. Antagonistic activity of yeasts against fungal pathogens.

The antagonistic test was conducted using the double culture method, as described by Limtong *et al.* [25] with modifications. A fungal plug (~6 mm diameter) was placed at the edge of a PDA plate. A 20 µL yeast suspension was streaked along a 5 cm section of the plate's edge and incubated for 48 hours. A second fungal plug was placed on the opposite side, 3 cm from the yeast streak, and then incubated at 29°C for seven days, with control plates containing only the fungal isolate. The diameter of the fungal colony was measured both horizontally and vertically. The percentage of fungi growth inhibition was calculated as follows:

$$Growth\ Inhibition\ (\%) = \frac{R_1 - R_2}{R_1} x\ 100\% \qquad (3)$$

where R_1 is the radius of the fungal colony in the control and R_2 is the radius of the fungal colony in the yeast-treated sample.

2.7. Volatile Compound activity of yeast isolates against fungal pathogens.

The volatile compound activity test against fungal pathogens was conducted using the two-Petri dish method, following the protocol of Parafati *et al.* [31]. A fungal plug (~6 mm in diameter) was excised using a sterile straw and inoculated onto PDA medium. In a separate Petri dish containing PDA, a well (~6 mm in diameter) was created using a sterile straw, onto which 20 µL of yeast suspension (at a cell density of 10^7 cells/mL) was inoculated. The Petri dish containing the fungal inoculum was then inverted and placed on top of the Petri dish containing the yeast suspension. The two Petri dish sections were sealed together with plastic wrap, ensuring that the fungal inoculum remained on the top, and the setup was incubated at 29°C for seven days. The control group consisted of fungal pathogens that received no yeast treatment. Fungal growth was assessed by measuring the diameter of the colony in both horizontal and vertical directions. The inhibition of fungal growth by volatile compounds was calculated as the percentage of inhibition (I) using the following formula [31]:

$$I = \frac{(C - T)}{C}\ x\ 100\% \qquad (4)$$

Where C is the diameter of the fungal colony in the control and T is the diameter of the fungal colony in the yeast treatment.

2.8. Data collection and statistical analysis.

Quantitative data from antagonistic and volatile compound inhibition assays were analysed using one-way Analysis of Variance (ANOVA) to determine significant differences between yeast treatments against each fungal pathogen. When significant differences were detected ($p < 0.05$), Duncan's Multiple Range Test (DMRT) was applied as a post-hoc test to distinguish between treatment means. Statistical analyses were performed using SPSS version 20 (IBM Corp., Armonk, NY, USA). Results are expressed as mean ± standard deviation (SD), and significant differences are indicated in the figures and tables using different superscript letters.

3. Results and Discussion

3.1. Results.

3.1.1. Fungi isolates from rotten cocoa pods.

A total of 37 fungal pathogens were successfully isolated from four cocoa pods exhibiting symptoms of pod rot (Figure 1). These symptoms included the presence of darkened or blackened areas on the pod surface, indicative of fungal infection. Over time, the affected pods developed a distinct, white, powdery layer composed of fungal spores. This visual manifestation is a key characteristic of cocoa pod rot, as described by Evans [32]. The blackened appearance of the fruit is primarily due to the necrosis of plant tissues caused by fungal colonisation. As the infection progresses, a characteristic white, powdery layer becomes evident on the surface of the affected pods, which is produced by the growth of fungi mycelium and spore formation, including various structures such as conidia, leading to the degradation of cellular structures within the pod and contributing significantly to the spread of infection across cocoa plantations [33].

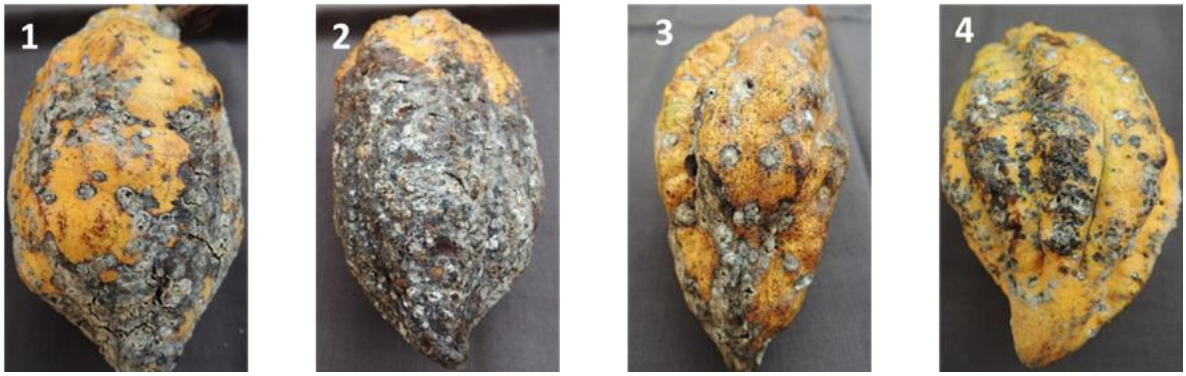


Figure 1. Samples of cocoa fruit showing symptoms of fruit rot: (**1**) Cocoa fruit 1; (**2**) Cocoa fruit 2; (**3**) Cocoa fruit 3; and (**4**) Cocoa fruit 4.

The isolation of fungal species from cocoa pod rot revealed a diverse array of colony characteristics (Figure 2). The identified morphological traits in this study include white colonies (11 isolates), pink mycelial colonies (15 isolates), yellowish colonies (8 isolates), and grey colonies (4 isolates). Each characterised colony type may correspond to different fungal

species or strains, which have been shown to exhibit diverse morphological characteristics, depending on the genus and environmental conditions. This morphological diversity is crucial for understanding the fungal pathogens responsible for cocoa pod rot and can help in assessing their potential impact on the cocoa industry [34,35].

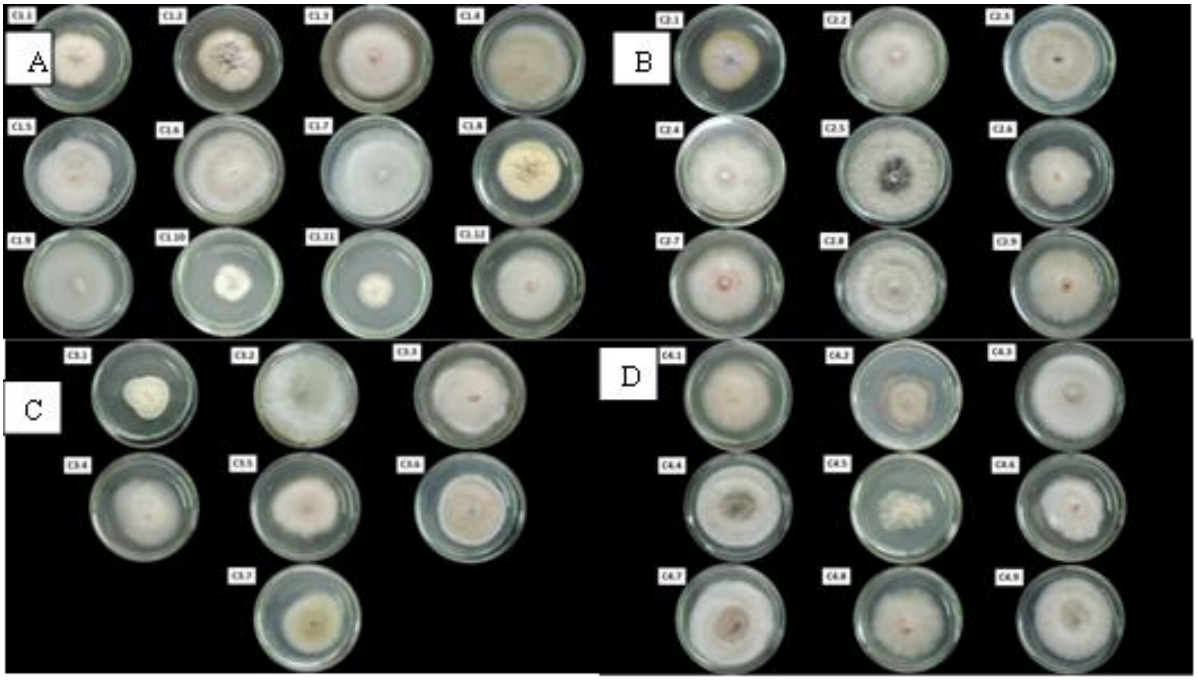
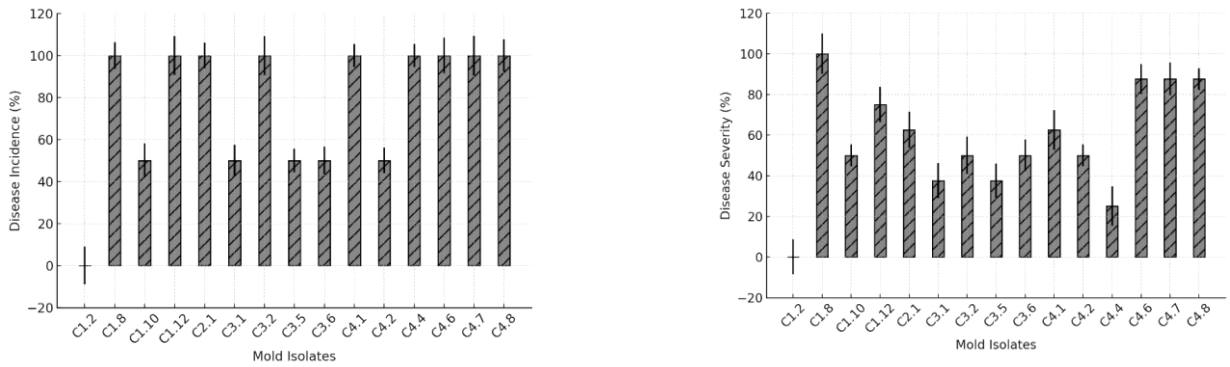


Figure 2. Fungi isolates obtained from cocoa pod rot; (**A**) Isolates from cocoa fruit 1; (**B**) Isolates from cocoa fruit 2; (**C**) Isolates from cocoa fruit 3; and (**D**) Isolates from cocoa fruit 4.

3.1.2. Pathogenicity of fungal isolates from rotten cocoa pods.

Fifteen selected fungal isolates were used for the pathogenicity test and were designated as C1.2, C1.8, C1.10, C1.12, C2.1, C3.1, C3.2, C3.5, C3.6, C4.1, C4.2, C4.4, C4.6, C4.7, and C4.8. The results demonstrated that these isolates exhibited varying pathogenic characteristics, as indicated by differences in disease incidence and disease severity values (Figure 3). Among them, nine isolates showed the highest disease incidence, reaching 100%. On the 8th day of incubation, infected cocoa pods exhibited signs of decay, characterised by a blackened fruit surface and the presence of white, floury fungi growth. Additionally, a greyish fungi mycelium was observed on the fruit stalk (Figure 4). In contrast, one isolate did not induce any disease symptoms.



(a) **(b)**

Figure 3. Pathogenicity test results of fungi isolates from rotten cocoa pods after an 8-day incubation at 29°C: **(a)** Disease Incidence (%); **(b)** Disease Severity (%).

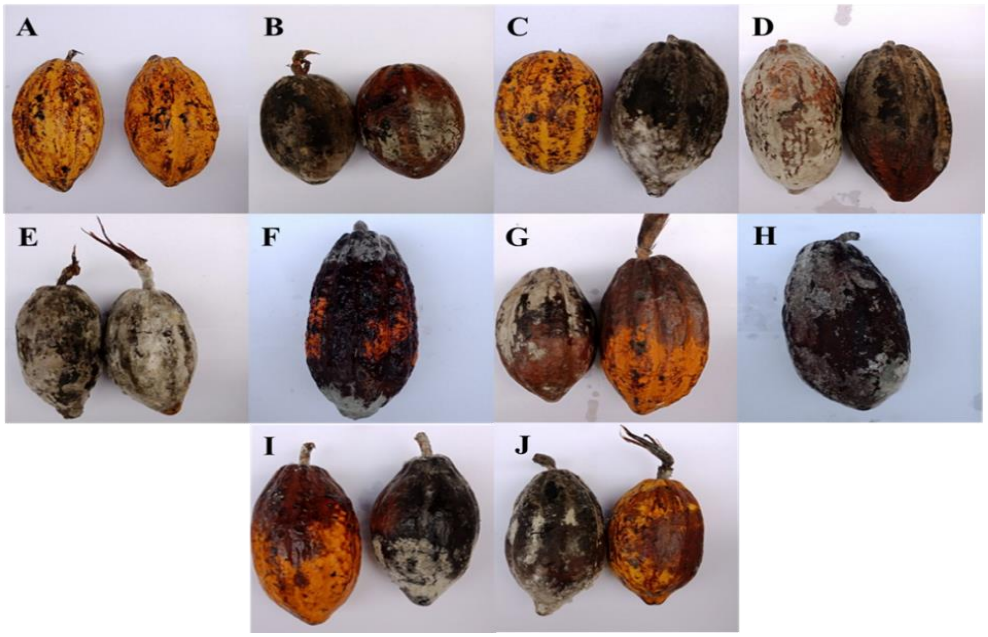


Figure 4. Cocoa pods inoculated with fungi isolates on the 8th day of incubation at 29°C: **(A)** Control; **(B)** Isolate C4.7; **(C)** Isolate C3.6; **(D)** Isolate C1.8; **(E)** Isolate C4.6; **(F)** Isolate C3.5; **(G)** Isolate C4.8; **(H)** Isolate C4.2; **(I)** Isolate C1.12; **(J)** Isolate C2.1.

The fungal isolate C1.8 exhibited the highest disease severity, reaching a score of 100%. Additionally, three fungi isolates, C4.6, C4.7, and C4.8, had a disease severity percentage of 87.5%. On the 8th day of incubation, the infected cocoa pods exhibited extensive rot, covering the entire surface, characterised by black discolouration and the presence of white, powdery fungal growth. While fungal spores spread from one of the inoculation points, they did not uniformly cover the entire fruit surface. Molecular identification was subsequently performed on isolates C1.8 (UNJCC F9), C4.7 (UNJCC F14), and C4.8 (UNJCC F18), as these isolates exhibited the highest disease severity.

3.1.3. Molecular identification of the yeasts and fungal isolates from rotten cocoa pods.

The yeast isolates used in this study were obtained from the Universitas Negeri Jakarta Culture Collection (UNJCC) and derived from cocoa bean fermentation. These isolates were designated as UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, and UNJCC Y-161. PCR amplification yielded nucleotide sequences with lengths of 533 bp, 616 bp, 571 bp, and 569 bp for isolates UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, and UNJCC Y-161, respectively.

Based on sequence alignment and phylogenetic analysis, UNJCC Y-158 was identified as *Rhodotorula alborubescens*, UNJCC Y-159 as *Meyerozyma guilliermondii*, and both UNJCC Y-160 and Y-161 as *Pichia kudriavzevii*. Phylogenetic tree analysis and construction were performed using MEGA 10 software, employing the Neighbour-Joining method with 1,000 bootstrap replications, following the approach described by Lestari *et al*. [36]. The results of the phylogenetic tree construction, with *Acarospora tintickiana* as the outgroup, indicated that yeast isolate UNJCC Y-158 clustered in the same clade as *Rhodotorula alborubescens* with a bootstrap value of 83%. Yeast isolate UNJCC Y-159 grouped with *Meyerozyma guilliermondii* with a bootstrap value of 97%. In contrast, yeast isolates UNJCC Y-160 and UNJCC Y-161 were positioned within the same clade as *Pichia kudriavzevii* with a bootstrap value of 99% (Figure 5).

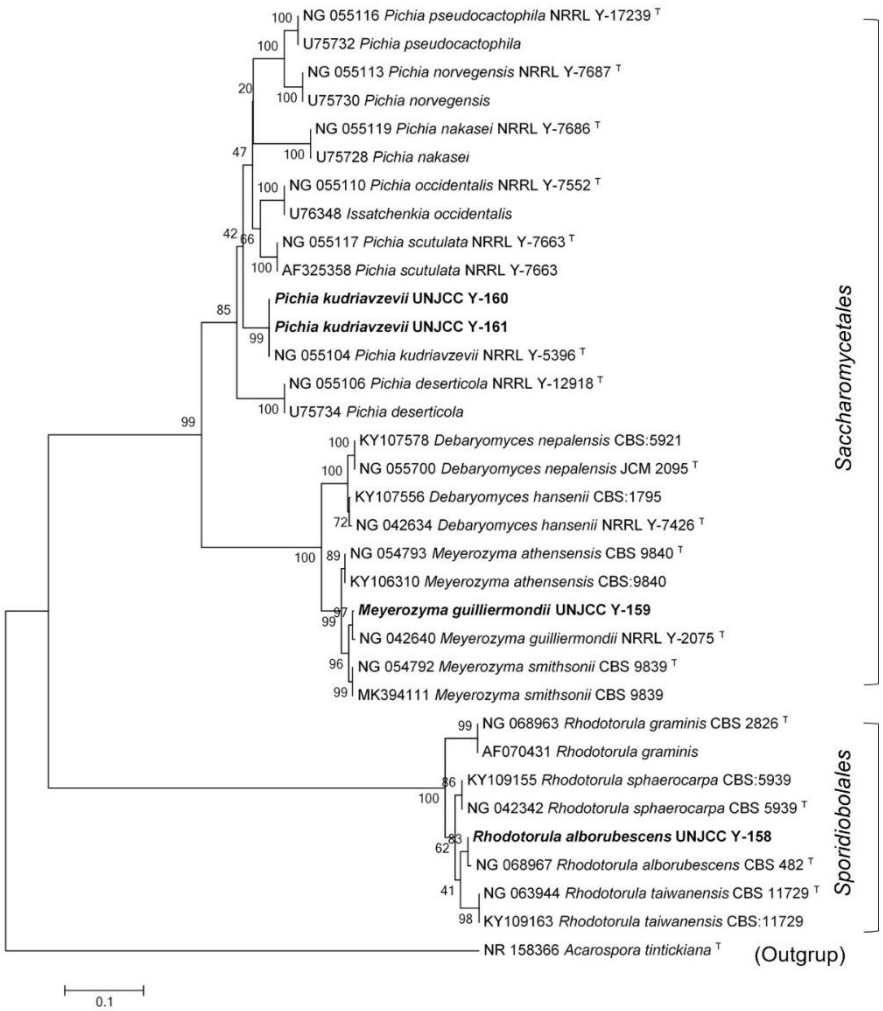


Figure 5. Phylogenetic tree of yeast isolates UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, and UNJCC Y-161, constructed based on the D1/D2 rDNA region analysis using the Neighbour-Joining method with 1,000 bootstrap replicates.

In this study, fungi isolates originating from rotten cocoa pods were obtained from the UNJCC collection. Based on sequence similarity and phylogenetic clustering, the isolates were identified as follows: UNJCC F9 as *Fusarium decemcellulare*, UNJCC F18 as *Fusarium solani*, and UNJCC F14 as *Colletotrichum siamense.* The selected isolates, UNJCC F9, UNJCC F18, and UNJCC F14, which demonstrated the highest severity in the pathogenicity tests, were subjected to molecular identification. The nucleotide sequence lengths for isolates UNJCC F9, UNJCC F18, and UNJCC F14 were 599 bp, 583 bp, and 578 bp, respectively. The results of the phylogenetic tree construction revealed that isolate UNJCC F9 clustered in the same clade as *Fusarium decemcellulare*, with a bootstrap value of 100% (Figure 6). In comparison, the isolate UNJCC F18 grouped with *Fusarium solani* (with a bootstrap value of 87%) (Figure 6). Isolate UNJCC F14 was positioned in the same clade as *Colletotrichum siamense*, with a bootstrap value of 95% (Figure 7). Bootstrap values between 70% and 100% indicate a high level of confidence in the phylogenetic tree, as reported by Simpson [37].

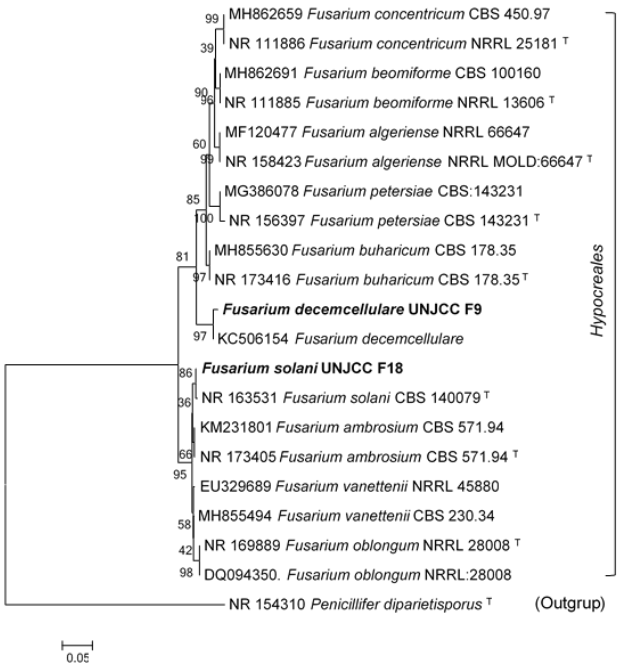


Figure 6. Phylogenetic tree of UNJCC F9 and UNJCC F18 fungi isolates based on ITS rDNA region analysis using the Neighbour-Joining method with 1,000 bootstrap replicates.

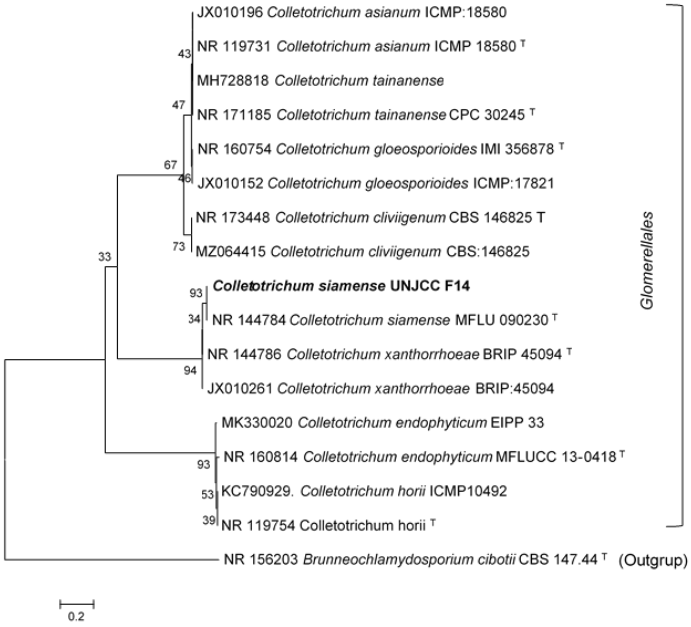


Figure 7. Phylogenetic tree of the UNJCC F14 fungi isolate based on ITS r-DNA region analysis using the Neighbour-Joining method with 1,000 bootstrap replicates.

3.1.4. Antagonistic activity of yeasts against fungal pathogens.

Antagonistic testing was conducted on four yeast isolates, UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, and UNJCC Y-161, against three fungal isolates: *Fusarium decemcellulare* UNJCC F9, *Fusarium solani* UNJCC F18, and *Colletotrichum siamense* UNJCC F14. The results of the analysis showed that the four yeast isolates significantly affected the inhibition of the tested fungi pathogens, with a significance value of $0.00 < \alpha$ (0.05). Figure 8 depicts the results of the antagonistic test on yeast isolates against *Fusarium decemcellulare* UNJCC F9, *Fusarium solani* UNJCC F18, and *Colletotrichum siamense* UNJCC F14 after 7 days of incubation. Overall, among the yeast isolates tested, *Pichia*

kudriavzevii UNJCC Y-160 demonstrated the highest inhibition percentage, with strong antagonistic activity against the fungal pathogens. In contrast, *Rhodotorula alborubescens* UNJCC Y-158 exhibited the lowest inhibition, suggesting minimal impact on fungal growth.

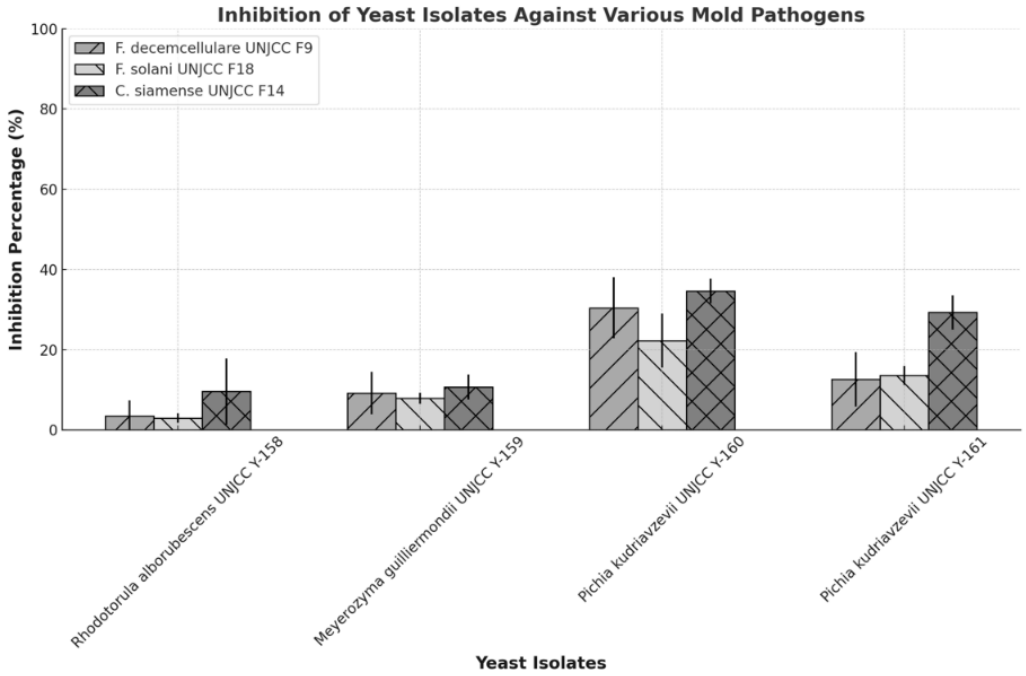


Figure 8. Comparison of antagonistic activity value between four yeast isolates against three fungi pathogens: *Fusarium* decemcellulare UNJCC F9, *Fusarium solani* UNJCC F18, and *Colletotrichum siamense* UNJCC F14 after 7 days of incubation.

Pichia kudriavzevii UNJCC Y-160 demonstrated the highest inhibition, with an inhibition percentage of 30.45% ± 5.74 against *Fusarium decemcellulare* UNJCC F9, indicating a strong antagonistic effect. In contrast, *Rhodotorula alborubescens* UNJCC Y-158 and *Meyerozyma guilliermondii* UNJCC Y-159 exhibited much lower inhibition, with values of 3.53% ± 3.01 and 9.22% ± 2.63, respectively. These lower inhibition percentages suggest that these yeast isolates are less effective in combating the *Fusarium decemcellulare* UNJCC F9. *Pichia kudriavzevii* UNJCC Y-161 showed moderate inhibition, with an inhibition percentage of 12.61% ± 1.86. Figure 9 shows the microscopic and macroscopic observations of the antagonistic test against *Fusarium decemcellulare* UNJCC F9 on the 7th day of incubation in PDA medium.

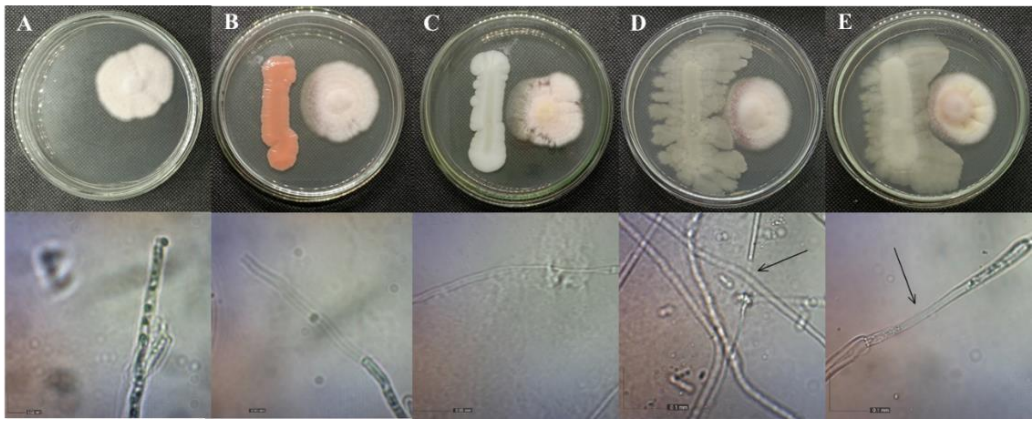


Figure 9. Macroscopic and microscopic observation of antagonistic activity tests of yeast isolates: **(A)** Control, **(B)** UNJCC Y-158; **(C)** UNJCC Y-159; **(D)** UNJCC Y-160; **(E)** UNJCC Y-161 against *Fusarium decemcellulare* UNJCC F9 on the 7th day of incubation in PDA medium (magnification 1000x).

Based on Duncan's test at the 5% significance level, the inhibition percentage of *Pichia kudriavzevii* UNJCC Y-160 was significantly different from *Rhodotorula alborubescens* UNJCC Y-158, *Meyerozyma guilliermondii* UNJCC Y-159, and *Pichia kudriavzevii* UNJCC Y-161. This indicates that *Rhodotorula alborubescens* UNJCC Y-158, *Meyerozyma guilliermondii* UNJCC Y-159, and *Pichia kudriavzevii* UNJCC Y-161, which did not differ statistically, exhibited similar inhibitory activity against *Fusarium decemcellulare* UNJCC F9.

Pichia kudriavzevii UNJCC Y-160 exhibited the highest percentage of inhibition against *Fusarium solani* UNJCC F18, with an inhibition value of 22.26% ± 6.74. In comparison, *Meyerozyma guilliermondii* UNJCC Y-159 showed lower inhibition (7.87%±1.39) than *Pichia kudriavzevii* UNJCC Y-161 (13.61% ± 2.38) (Figure 8). This finding is consistent with the study by Madbouly *et al.* [38], which reported that *Meyerozyma guilliermondii* inhibited *Fusarium cerealis* M3, *Fusarium graminearum* M25, and *Fusarium poae* with inhibition percentages of 66.7%, 88.3%, and 79.8%, respectively. These values are lower than the inhibition reported for *Pichia kudriavzevii*, which showed inhibition rates of 88.6%, 91.6%, and 85.4% against similar fungi strains. *Rhodotorula alborubescens* UNJCC Y-158, on the other hand, showed only 2.93% ± 1.22 inhibition against *Fusarium solani* UNJCC F18.

Microscopic observations of the antagonistic test revealed the attachment of yeast cells to the hyphae of *Fusarium solani* UNJCC F18 (Figure 10), leading to noticeable changes in the fungal hyphae. Sharma *et al.* [39] found that the attachment of yeast cells to fungal hyphae facilitates the yeast's nutrient absorption, allowing it to utilise the fungal hyphae for growth. Additionally, the activity of hydrolytic enzymes, such as cellulase, may contribute to the degradation of the fungi's cell wall, further inhibiting its growth.

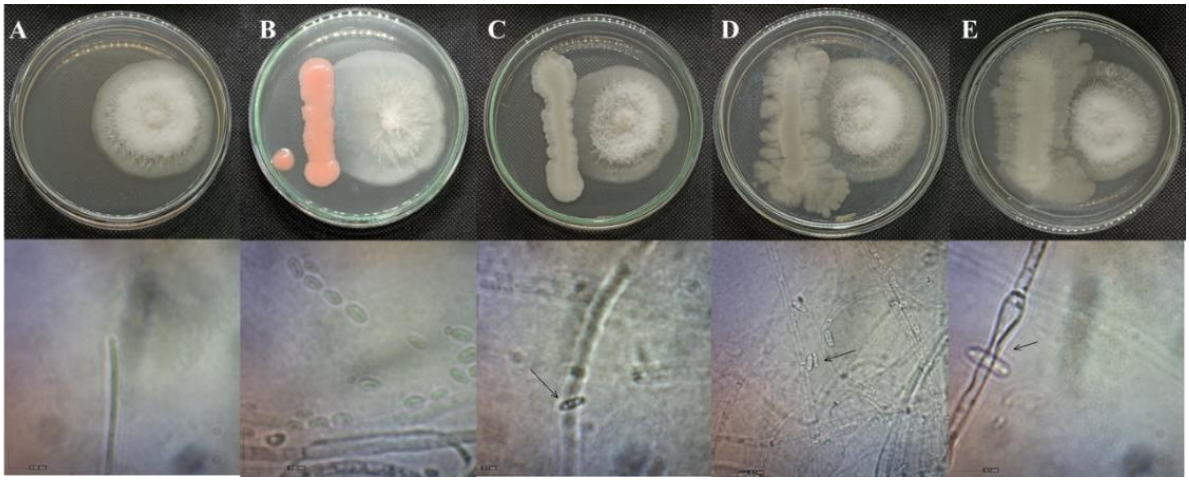


Figure 10. Macroscopic and microscopic observation of antagonistic activity tests of yeast isolates: **(A)** Control, **(B)** UNJCC Y-158; **(C)** UNJCC Y-159; **(D)** UNJCC Y-160; **(E)** UNJCC Y-161 against *Fusarium solani* UNJCC F18 on the 7th day of incubation in PDA medium (magnification 1000x)

Pichia kudriavzevii UNJCC Y-160 exhibited the highest inhibition of *Colletotrichum siamense* UNJCC F14. At the same time, *Rhodotorula alborubescens* UNJCC Y-158 and *Meyerozyma guilliermondii* UNJCC Y-159 showed less inhibition, as confirmed by both visual and microscopic observations (Figure 11). *Rhodotorula alborubescens* UNJCC Y-158 exhibited an inhibition of 9.58% ± 8.22 against *Colletotrichum siamense* UNJCC F14, while *Meyerozyma guilliermondii* UNJCC Y-159 showed a slightly higher inhibition of 10.68% ± 3.11 (Figure 8). Neither isolate differed significantly from the other. In contrast, *Pichia kudriavzevii* UNJCC Y-160 and *Pichia kudriavzevii* UNJCC Y-161 exhibited higher inhibition percentages against *Colletotrichum siamense* UNJCC F14 compared to *Rhodotorula*

alborubescens UNJCC Y-158 and *Meyerozyma guilliermondii* UNJCC Y-159 (Figure 8). *Pichia kudriavzevii* UNJCC Y-160 showed the highest inhibition with 34.64% ± 3.06, significantly outperforming the other isolates. *Pichia kudriavzevii* UNJCC Y-161 demonstrated a moderate inhibition of 29.27% ± 4.22, which was not significantly different from either *Pichia kudriavzevii* UNJCC Y-160 or the other yeast isolates.

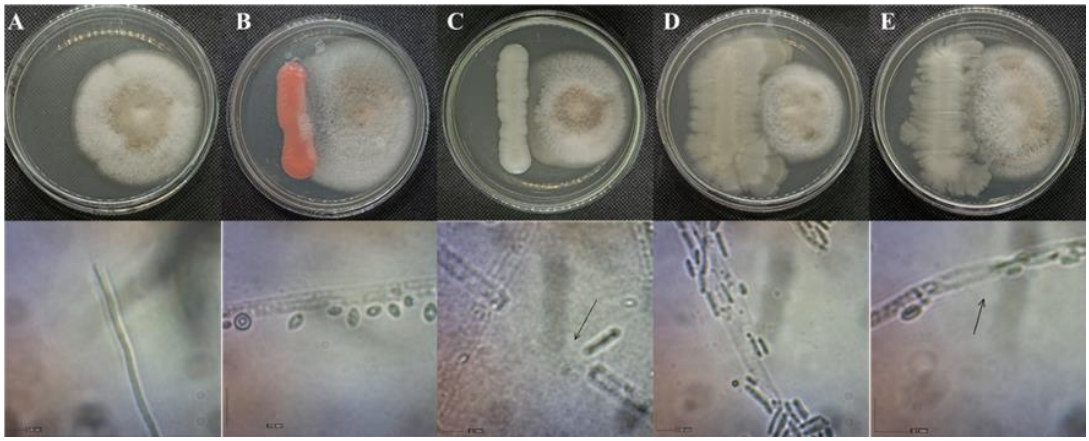


Figure 11. Macroscopic and microscopic observation of antagonistic activity tests of yeast isolates: **(A)** Control, **(B)** UNJCC Y-158; **(C)** UNJCC Y-159; **(D)** UNJCC Y-160; **(E)** UNJCC Y-161 against *Colletotrichum siamense* UNJCC F14 on the 7th day of incubation in PDA medium (magnification 1000x).

According to Bleve *et al.* [40], *Pichia kudriavzevii* 17C2 and 16C2 yeasts were able to suppress the growth of *Aspergillus carbonarius* and *A. niger*, achieving 100% inhibition in *in vitro* tests, which was attributed to the production of secondary metabolites and nutrient competition. Similarly, Chanchaichaovivat *et al.* [41] reported that several yeast species, including *Pichia guilliermondii*, *Candida musae*, *Issatchenkia orientalis*, and *Clavispora quercitrusa*, were effective in reducing the incidence of anthracnose disease in chilli fruits caused by *Colletotrichum capsici*.

3.1.5. Volatile compounds activity of yeast isolates from cocoa bean fermentation against pathogenic fungi.

The volatile compound inhibition test was performed on four yeast isolates, UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, and UNJCC Y-161, against the three pathogenic fungi: *Fusarium decemcellulare* UNJCC F9, *Fusarium solani* UNJCC F18, and *Colletotrichum siamense* UNJCC F14. Figure 12 represents the comparison of volatile compound activity values between the yeast isolates, as indicated by the inhibition percentage obtained after 7 days of incubation. The yeast isolates *Pichia kudriavzevii* UNJCC Y-160 and *Pichia kudriavzevii* UNJCC Y-161 showed the highest inhibition, particularly against *Fusarium decemcellulare* UNJCC F9, with significant inhibition percentages exceeding 40%. *Rhodotorula alborubescens* UNJCC Y-158 and *Meyerozyma guilliermondii* UNJCC Y-159 displayed relatively lower inhibition percentages, particularly against *F. decemcellulare* UNJCC F9, as shown by the error bars indicating variability (Table 1).

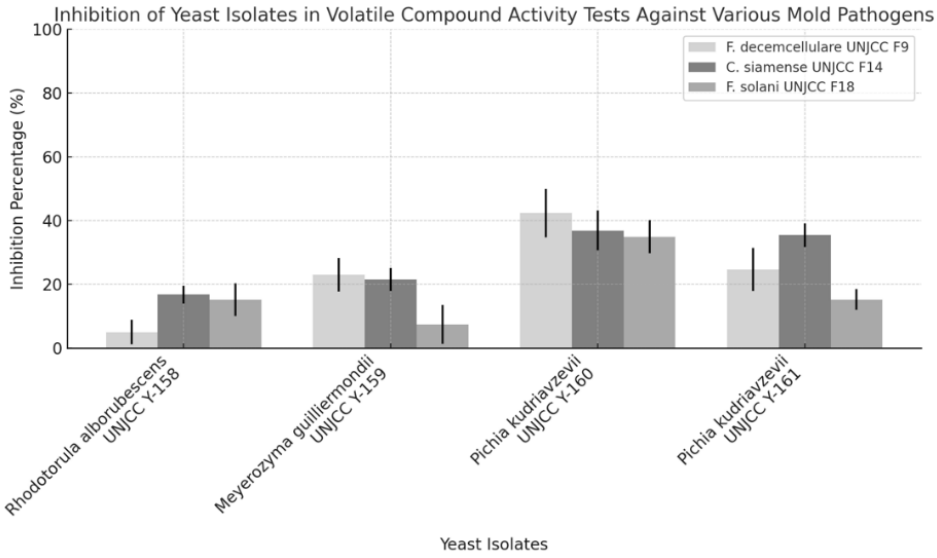


Figure 12. Comparison of volatile compound activity between the yeast isolates against three fungi pathogens: *Fusarium decemcellulare* UNJCC F9, *Fusarium solani* UNJCC F18, and *Colletotrichum siamense* UNJCC F14 after 7 days of incubation in PDA media.

Table 1. Percent inhibition of three cocoa pod fungal pathogens (Fusarium decemcellulare, Fusarium solani, and Colletotrichum siamense) by yeast isolates (Rhodotorula alborubescens, Meyerozyma guilliermondii, and Pichia kudriavzevii) through antagonistic interaction and volatile organic compound (VOC) production.

Yeast isolate	Pathogen	% Inhibition (mean ± SD)	Inhibition level	VOC % inhibition (mean ± SD)
UNJCC Y-158 (*R. alborubescens*)	*F. decemcellulare*	3.53 ± 3.01	Weak	5.01 ± 3.80
UNJCC Y-159 (*M. guilliermondii*)	*F. decemcellulare*	9.22 ± 2.63	Weak	22.94 ± 5.27
UNJCC Y-160 (*P. kudriavzevii*)	*F. decemcellulare*	30.45 ± 5.74	Moderate	42.37 ± 7.60
UNJCC Y-161 (*P. kudriavzevii*)	*F. decemcellulare*	12.61 ± 1.86	Weak	24.66 ± 6.78
UNJCC Y-158 (*R. alborubescens*)	*F. solani*	2.93 ± 1.22	Weak	15.18 ± 5.05
UNJCC Y-159 (*M. guilliermondii*)	*F. solani*	7.87 ± 1.39	Weak	7.39 ± 6.06
UNJCC Y-160 (*P. kudriavzevii*)	*F. solani*	22.26 ± 6.74	Moderate	34.94 ± 5.13
UNJCC Y-161 (*P. kudriavzevii*)	*F. solani*	13.61 ± 2.38	Weak	15.24 ± 3.27
UNJCC Y-158 (*R. alborubescens*)	*C. siamense*	9.58 ± 8.22	Weak	16.76 ± 2.81
UNJCC Y-159 (*M. guilliermondii*)	*C. siamense*	10.68 ± 3.11	Weak	21.49 ± 3.60
UNJCC Y-160 (*P. kudriavzevii*)	*C. siamense*	34.64 ± 3.06	Moderate	36.88 ± 6.27
UNJCC Y-161 (*P. kudriavzevii*)	*C. siamense*	29.27 ± 4.22	Moderate	35.40 ± 3.73

The one-way ANOVA on the percentage of inhibition of *Fusarium decemcellulare* UNJCC F9 indicated that the four yeast isolates had a significant effect on the inhibition percentage, with a p-value of 0.02, which is less than the significance level of 0.05. According to the results from the Duncan test at the 5% level, the inhibition percentage of *Pichia kudriavzevii* UNJCC Y-160 was not significantly different from the average inhibition of *Meyerozyma guilliermondii* UNJCC Y-159 and *Pichia kudriavzevii* UNJCC Y-161. However, it was substantially different from *Rhodotorula alborubescens* UNJCC Y-158. *Pichia kudriavzevii* UNJCC Y-160 exhibited the highest inhibition at 42.37% ± 7.60, followed by *Pichia kudriavzevii* UNJCC Y-161 with 24.66% ± 6.78 (Figure 12). *Rhodotorula alborubescens* UNJCC Y-158 showed the lowest inhibition at 5.01% ± 3.80, with *Meyerozyma guilliermondii* UNJCC Y-159 exhibiting a moderate inhibition of 22.94% ± 5.27. The volatile compounds produced by the four UNJCC yeast isolates induced changes in the pigmentation of the *Fusarium decemcellulare* UNJCC F9, as seen in Figure 13.

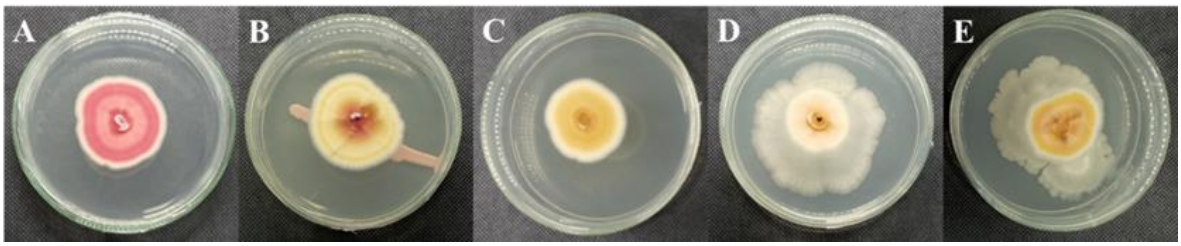


Figure 13. Macroscopic observation of the volatile compound activity test of the yeast isolates: **(A)** Control; **(B)** UNJCC Y-158; **(C)** UNJCC Y-159; **(D)** UNJCC Y-160; **(E)** UNJCC Y-161 against *Fusarium decemcellulare* UNJCC F9 on the 7th day of incubation in PDA media.

The one-way ANOVA on the percentage of inhibition of *Fusarium solani* UNJCC F18 revealed that the four yeast isolates had a significant effect on the inhibition percentage, with a p-value of 0.01, which is less than the significance level of $\alpha = 0.05$. Among the isolates, *Pichia kudriavzevii* UNJCC Y-160 exhibited the highest inhibition at 34.94% ± 5.13, followed by *Pichia kudriavzevii* UNJCC Y-161 with 15.24% ± 3.27 (Figure 12). *Rhodotorula alborubescens* UNJCC Y-158 and *Meyerozyma guilliermondii* UNJCC Y-159 showed lower inhibition, with values of 15.18% ± 5.05 and 7.39% ± 6.06, respectively. The macroscopic observation of the volatile compound activity of the four yeast isolates against *Fusarium solani* UNJCC F18 is shown in Figure 14 and Table 1.

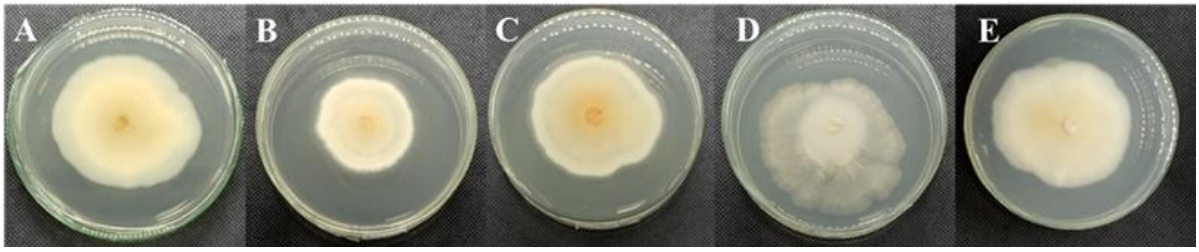


Figure 14. Macroscopic observation of the volatile compound activity test of the yeast isolates: **(A)** Control; **(B)** UNJCC Y-158; **(C)** UNJCC Y-159; **(D)** UNJCC Y-160; **(E)** UNJCC Y-161 against *Fusarium solani* UNJCC F18 on the 7th day of incubation in PDA media.

The one-way ANOVA on the inhibition percentage of *Colletotrichum siamense* UNJCC F14 indicated a significant effect of the four yeast isolates, with a p-value of 0.02, which is less than the significance level α (0.05). The results show that *Pichia kudriavzevii* UNJCC Y-160 exhibited the highest inhibition percentage (36.88% ± 6.27%), which was significantly higher than *Rhodotorula alborubescens* UNJCC Y-158 (16.76% ± 2.81%) and *Meyerozyma guilliermondii* UNJCC Y-159 (21.49% ± 3.60%).

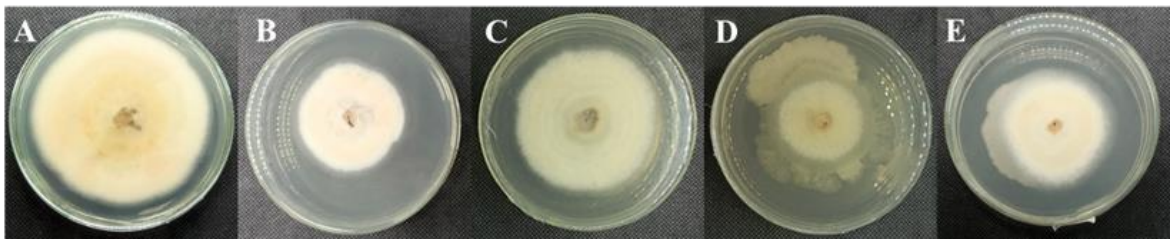


Figure 15. Macroscopic observation of the volatile compound activity test of the yeast isolates: **(A)** Control; **(B)** UNJCC Y-158; **(C)** UNJCC Y-159; **(D)** UNJCC Y-160; **(E)** UNJCC Y-161 against *Colletotrichum siamense* UNJCC F14 on the 7th day of incubation in PDA media.

Additionally, *Pichia kudriavzevii* UNJCC Y-161 showed moderate inhibition (35.40% ± 3.73%), which was not significantly different from *Pichia kudriavzevii* UNJCC Y-160 but

was significantly different from the other yeast isolates. The macroscopic observation of the volatile compound activity of the four yeast isolates against *Colletotrichum siamense* UNJCC F14 is shown in Figure 15.

3.2. Discussion.

Cocoa plants (*Theobroma cacao* L.) are a crucial plantation commodity that plays a significant role in increasing the income and welfare of farmers, particularly in cocoa development centres in Indonesia. However, the problem of cocoa diseases is becoming increasingly prominent due to the complex interaction of environmental factors, pathogen presence, and cultivation practices [42]. Cocoa plants, like many crops, are susceptible to a variety of diseases caused by fungi, bacteria, and viruses, with fungal infections being among the most devastating. These diseases not only affect the yield and quality of cocoa beans but also significantly impact the livelihood of farmers, particularly in tropical regions where cocoa is a major commodity [43].

One of the primary challenges in cocoa plantations is the spread of fungal pathogens. In this study, we have successfully isolated fungal pathogens from rotten cocoa fruits, which exhibited a blackish colour with the appearance of a white, floury layer, as described by Evans [32]. The pathogenic fungi obtained from these rotten cocoa fruits have distinct macroscopic characteristics: white colonies (11 isolates), pink mycelial colonies (15 isolates), yellowish colonies (8 isolates), and grey colonies (4 isolates). These morphological characteristics of the fungi are similar to those of fungi in the genus *Fusarium* [44,45].

The results of the molecular identification of selected fungal isolates (UNJCC F9, UNJCC F18, and UNJCC F14), which demonstrated the highest disease incidence (DI) and disease severity (DS) in the pathogenicity tests, revealed similar genetic characteristics to those of *Fusarium decemcellulare*, *Fusarium solani*, and *Colletotrichum siamense*, respectively. These pathogens have been reported to cause diseases in cocoa by invading the internal tissues, resulting in the cocoa beans rotting and leading to discolouration and decay [5, 46-48]. *Fusarium decemcellulare* has specifically been identified as the causal agent of cushion gall disease in cocoa, characterised by internal tissue degradation resulting in blackened areas [44]. Similarly, research has demonstrated that *Colletotrichum siamense* can cause disease in cocoa fruits, leading to internal tissue damage and blackening [49].

In this study, symptoms of fruit rot began to appear between the 4th and 8th day, characterised by blackish spots and visible mycelium on the fruit surface. Research by Hafsah and Zuyasna [50] demonstrated that fungi isolates could induce pathogenic symptoms in cocoa fruit, with lesion diameters ranging from 2.39 to 3.64 cm within 3 days of inoculation. The average incubation period ranged from 2.11 to 3.06 days, and the highest percentage of symptom appearance was between 75% and 99%. In addition, a recent study by Perrine-Walker [47] reported that the initial symptoms of fruit rot disease caused by *Phytophthora* were marked by the appearance of spots on the surface of the fruit within 2–3 days after infection, followed by browning. These symptoms continued to spread and covered the entire surface between the 7th and 14th days under humid conditions.

Research has demonstrated that relative humidity (RH) significantly influences the germination and sporulation of *Fusarium* spp., which are among the fungi affecting cocoa. For instance, studies have shown that higher RH levels enhance the production of spores in *Fusarium oxysporum*, a common soil-borne pathogen [51]. Furthermore, the movement of *Fusarium oxysporum* propagules through soil is affected by water percolation, which can

disperse the pathogen from the soil into the root zone of cocoa plants. Similarly, *Fusarium decemcellulare*, another pathogen affecting cocoa, has been associated with dieback diseases, with its development influenced by environmental conditions [45].

Pathogenic fungi that infect cocoa fruits can originate from various sources, including soil and diseased plants. Once these fungi infect the fruit, they produce numerous sporangia, reproductive spores that are dispersed by water and wind [51]. This dispersal mechanism allows the infection to reach even fruits located high on the tree. The infection typically progresses from the stalk to the stem and eventually to the fruit. Environmental factors, particularly moderate temperature and high humidity, play a significant role in this process by promoting spore formation and increasing the risk of infection [52].

In response to the challenges posed by these diseases, integrated disease management strategies have been proposed, combining chemical, biological, and cultural control methods to address the issue. The use of biocontrol agents, such as specific strains of yeast, has gained attention as a sustainable and eco-friendly alternative to chemical fungicides. These yeasts can inhibit the growth of harmful fungi through mechanisms such as competition for space and nutrients, the production of hydrolytic enzymes, and the release of volatile compounds [53]. Yeast can grow quickly, thrive in unfavourable conditions, and does not produce mycotoxins or allergenic spores [54]. Many studies have shown that yeast is highly effective in inhibiting the growth of pathogenic fungi that cause post-harvest diseases in plants [55]. Research by Chen *et al.* [56] reported that *Rhodotorula alborubescens* Y1923 could inhibit the growth of *Botrytis cinerea*, which causes grey mould disease in apples. Meanwhile, *Meyerozyma guilliermondii* Y-1 has been shown to suppress Apple ring rot disease caused by *Botryosphaeria dothidea in vivo* [57].

The molecular identification of the four potential yeast isolates (UNJCC Y-158, UNJCC Y-159, UNJCC Y-160, and UNJCC Y-161) revealed that they share genetic similarities with *Rhodotorula alborubescens*, *Meyerozyma guilliermondii*, and *Pichia kudriavzevii* (Figure 5). These isolates demonstrated varying degrees of inhibition against the tested fungi. Fernandez-San Millan *et al.* [58] reported that inhibition levels against pathogenic fungi using the dual culture method can be categorised into four ranges: weak (0–25%), moderate (26–50%), strong (51–75%), and very strong (76–100%). This categorisation is reflected in the reduction of the fungal colony diameter when grown in the presence of yeast isolates. Based on this classification, *Rhodotorula alborubescens* UNJCC Y-158 and *Meyerozyma guilliermondii* UNJCC Y-159 exhibited weak inhibition, with values ranging from 2.93% to 10.68%. On the other hand, *Pichia kudriavzevii* UNJCC Y-160 and *Pichia kudriavzevii* UNJCC Y-161 demonstrated inhibition levels ranging from weak to moderate, with values between 12.61% and 34.64%. Sperandio [59] and Ruiz-Moyano [60] reported a reduction in fungi growth in the presence of yeast, likely due to the yeast's ability to suppress mycelium development. These findings are consistent with previous studies that utilised yeast as an antagonist agent, further supporting the potential of these yeast isolates in biocontrol applications.

The inhibition of yeast against fungal pathogens was likely due to competition for space and nutrients. This competitive mechanism can be observed through increased yeast growth in the medium, where the yeast cells dominate and expand, thereby outcompeting the fungi cells for nutrients. This phenomenon results in the fungi cells lacking the necessary resources to grow, leading to reduced mycelium formation and inhibition of fungal growth [16]. Muhibuddin and Sholihah [61] reported that when pathogenic fungi are inoculated alongside

yeast, they lack the nutrients and space necessary to grow, which prolongs their adaptation to the media. *Meyerozyma guilliermondii* has been shown to inhibit the growth of *Penicillium italicum* fungi through spatial and nutrient competition, a key mechanism in fungal inhibition, alongside the yeast's ability to produce antimicrobial compounds or metabolites [62].

From microscopic observations (Figures 9, 10, and 11), the phenomenon of hyperparasitism is visible. Hyperparasitism occurs when yeast cells directly interact with fungi hyphae, using them as hosts for their growth [39]. After 7 days of incubation, the interaction between yeast cells and fungal hyphae was examined under a microscope. Fungal hyphae near the yeast cells displayed significant morphological changes, including thinning, shortening, swelling, and shrinking. These alterations are likely caused by the activity of hydrolytic enzymes produced by the yeast, which degrade the fungi's cell wall and lead to changes in the hyphal structure [63]. All yeast isolates in this study were found to produce cellulase enzymes, which are crucial for the degradation of fungal hyphae [64].

Additionally, *Pichia kudriavzevii* has been reported to produce extracellular enzymes, such as chitinase, β-1,3-glucanase, and pectinase, which inhibit fungal growth [38]. Furthermore, yeast cells were observed attaching to the fungal hyphal cell walls, preventing the hyphae from absorbing essential nutrients. This attachment is critical in inhibiting fungal growth, as nutrient acquisition is vital for fungal growth and development.

The results of the volatile compound activity test showed that *P. kudriavzevii* UNJCC Y-160 and *P. kudriavzevii* UNJCC Y-161 produced significant inhibitory effects on the tested pathogens. Recent studies have demonstrated that yeast-derived volatile organic compounds (VOCs) possess a broad spectrum of antimicrobial activities, encompassing antifungal, antibacterial, and antiviral effects. For example, *Pichia kudriavzevii* has been found to produce inhibitory compounds that significantly reduce the growth of various pathogenic fungi, including *Penicillium chrysogenum* M24, *Aspergillus fumigatus*, and *Mucor* sp., with inhibition values of 91.3%, 90.1%, and 73.5%, respectively [65]. A more recent study also reported that *P. kudriavzevii* could reduce the diameter of *Penicillium glabrum* by 23.91% by releasing VOCs [66]. Additionally, *Saccharomyces cerevisiae* has been shown to release aldehyde-based VOCs that disrupt the cell walls of fungi like *Fusarium* and *Botrytis*, demonstrating the potential of these natural compounds in disease management [67].

VOCs are small carbon-based molecules that are volatile, easily evaporating due to their low water solubility and high vapour pressure [68,69]. Buzzini *et al.* [70] reported that Ascomycota yeasts, particularly those isolated from tropical regions, produce a variety of VOCs, including alcohols (e.g., amyl alcohol and isoamyl alcohol), aldehydes (e.g., 2-methyl-2-hexane and 2-isopropyl-5-methyl-2-hexane), and esters (e.g., ethyl isobutyrate, isoamyl acetate, and ethyl isovalerate). These VOCs play a critical role in inhibiting the growth of pathogenic fungi by disrupting several biological processes. Specifically, VOCs affect the biogenesis of the fungi's cell wall, which is primarily composed of mannoproteins, glucans, and chitin. By inhibiting the synthesis of these key components, VOCs compromise the structural integrity of the fungal cell wall, making it more susceptible to damage. This disruption reduces the fungi's ability to absorb essential nutrients, which ultimately impairs their growth and development [71]. Furthermore, VOCs released by yeasts can interact with fungi cells through various mechanisms, including the inhibition of sporulation, growth retardation, and the disruption of hyphal morphogenesis [68]. The production of VOCs by yeasts, therefore, represents a promising biocontrol strategy, reducing the reliance on chemical fungicides and enhancing sustainable agriculture practices.

In this study, several modes of action were observed in the antagonistic interaction between yeast isolates and fungal pathogens. Firstly, nutrient and space competition was apparent in the dual culture assays, where both yeast and fungi were placed on the same Petri dish without any physical separation. The observed suppression of fungal colony expansion near yeast streaks suggests that the yeasts outcompeted the fungi for nutrients and physical space. This is consistent with previous findings that effective nutrient competition can inhibit fungal development by depriving it of essential resources [61,62]. Secondly, the role of volatile organic compounds (VOCs was confirmed using the two-Petri dish method, which ensured that inhibition occurred without direct contact. Yeasts are known to produce a variety of VOCs such as higher alcohols (e.g., isoamyl alcohol), esters (e.g., ethyl acetate), aldehydes (e.g., acetaldehyde), and organic acids. These compounds interfere with spore germination, hyphal growth, and cell wall biosynthesis in pathogenic fungi [65-67]. In particular, *Pichia kudriavzevii* has been reported to reduce the diameter of *Penicillium glabrum* through VOCs and to inhibit multiple fungal species, including *Mucor* sp. and *Aspergillus fumigatus* [66]. Thirdly, hydrolytic enzymes played a significant role in disrupting fungal morphology. Microscopic observations revealed that yeast cells adhered to fungal hyphae, resulting in structural abnormalities, including thinning, shrinking, and swelling of the hyphal filaments. These symptoms are indicative of enzymatic degradation, particularly by enzymes such as β-1,3-glucanase, cellulase, and chitinase, which target fungal cell wall polysaccharides [6, 38, 39, 42. For example, *Pichia kudriavzevii* has been reported to produce extracellular enzymes that disrupt fungal structure and suppress pathogen development [38]. Together, these mechanisms, competition, VOC production, and enzymatic degradation highlight the multifaceted biocontrol potential of the yeast isolates studied here, supporting their applicability as sustainable alternatives to chemical fungicides.

4. Conclusions

Four out of seven yeast isolates demonstrated antagonistic activity against all tested fungal pathogens. The four yeast isolates *Rhodotorula alborubescens* UNJCC Y-158, *Meyerozyma guilliermondii* UNJCC Y-159, *Pichia kudriavzevii* UNJCC Y-160, and *Pichia kudriavzevii* UNJCC Y-161, which were isolated from cocoa bean fermentation, have demonstrated potential in inhibiting the growth of three pathogenic fungi: *Fusarium decemcellulare* UNJCC F9, *Fusarium solani* UNJCC F18, and *Colletotrichum siamense* UNJCC F14, which cause cocoa pod rot. These yeast isolates exhibited their inhibitory effects through various mechanisms, including antagonism, hyperparasitism, and the production of volatile compounds. The yeast-mediated inhibition of fungal growth can provide a promising alternative to conventional chemical fungicides, offering a more environmentally friendly and safer solution for managing cocoa diseases. The use of these yeast isolates as biocontrol agents offers significant advantages over the chemical treatments commonly employed in cocoa plantations. Fungicides, while effective, often pose risks to both human health and the environment, making yeast-based biocontrol a more sustainable option. Given the natural origin of these yeast isolates and their demonstrated efficacy against key cocoa pathogens, they represent a valuable tool for integrated pest management systems. Future research could further explore the scalability and practical application of these yeast isolates in real-world agricultural settings to help reduce the reliance on harmful chemicals while maintaining healthy cocoa production.

Author Contributions

Conceptualization, D.S., S.N.A.H., A.F., D.N.S., and R.H.B.S.; methodology, D.S., S.N.A.H., A.F., and D.N.S.; data curation, L.A., L.Ag., M.A.A., and H.A.E.E.; formal analysis, D.S., S.N.A.H., A.F., and D.N.S.; writing—original draft preparation, D.S., R.H.B.S., and L.A.; writing—review and editing, D.S. and R.H.B.S.; All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
VOCs	Volatile Organic Compounds
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
YMA	Yeast Malt Agar
DI	Disease Incidence
DS	Disease Severity

Abbreviation	Definition
UNJCC	Universitas Negeri Jakarta Culture Collection
ANOVA	Analysis of Variance
DMRT	Duncan's Multiple Range Test
PCR	Polymerase Chain Reaction
BLAST	Basic Local Alignment Search Tool Algorithm
NJ	Neighbor Joining

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