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RESEARCH ARTICLE

Antifungal Mechanism of *Rhodotorula mucilaginosa* and *Aureobasidium* sp. nov. Isolated from *Cerbera manghas* L. against the Growth of Destructive Molds in Post Harvested Apples

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Abstract: ***Background*:** Apples often experience postharvest damage due to being attacked by mold organisms. Several groups of molds such as *Aspergillus* sp., *Penicilium expansum, Botrytis cinerea*, and *Venturia* sp. can cause a serious postharvest disease exhibited as watery regions where areas of blue-green tufts of spores develop. Current methods using fungicides to control pathogenic fungi can cause resistance if applied in the long term. An alternative procedure using yeast as a biological agent has been found.

***Objective*:** The aim of this study is to screen potential yeast, which has the ability to inhibit the growth of *Aspergillus brasielensis* (isolate A1) and *Aspergillus flavus* section flavi (isolate A17) isolated from apple fruits.

Methods: Antagonism test using YMA dual culture medium using *in vitro* assays and ITS rDNA identification were performed.

Results: The result showed that 3 out of 19 yeast isolated from *Cerbera manghas* L, T1, T3 and T4, demonstrated the potential ability as a biocontrol agent. ITS rDNA identification demonstrated that T1 has a similarity to *Rhodotorula mucilaginosa* while T3 and T4 were identified as *Aureobasidium* sp. nov. The 3 isolates exhibited the ability to reduce the growth of *A. brasiliensis sensu lato* better than dithane 0.3% with a Disease Incidence (DI) of 100% and a Disease Severity (DS) value of 45%. Only isolate T1 and T3 were able to reduce decay symptoms in apples inoculated with *A. flavus sensu lato* (with DO and DS were 100% and 25%, respectively) compared to dithane pesticides 0.3%.

Conclusion: This study indicated that competition between nutrients occurs between pathogenic molds and under-yeast *in vitro* and *in vivo* conditions. However, further studies in the future might be able to elucidate the 'killer' activity and interaction with the pathogen cells and the bio-product production using *Rhodotorula mucilaginosa* and *Aureoubasidium namibiae* strains to control postharvest diseases.

Keywords: Apple, *Aureobasidium pullulans*, biocontrol fungi, *Rhodotorula mucilaginosa*, molds, fungicides.

1. INTRODUCTION

Apples are beneficial to consume by people since it is rich in vitamins A, B and C, and minerals such as calcium, phosphorus, iron, chlorine, magnesium, sodium and potassium [1, 2]. Based on the data from the Ministry of Agriculture Indonesia, apple production in 2013 was 255.33 tons, and it decreased to 242.91 tons in 2014 [3]. Several factors contributed to this decline, one of which is the presence of destructive microorganisms such as fungi that causes damage during postharvest production [4, 5]. Destructive molds such as *Aspergillus* sp., *Penicillium expansum*, *Botrytis cine-*

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rea, Colletotrichum acutatum, Monilia fructigena, Fusarium avenaceum, Mucor sp. and *Rhizopus stolonifer* [6-8] can cause damage to postharvest fruit, especially in apples. *B. cinerea*, for instance, causes gray mold rot (gray mold rot) on postharvest apples [9], while *P. expansum* causes blue mold rot in postharvest apples and also produces mycotoxin patulin and enzyme [10].

Harvest and handling practices have major effects on postharvest decay. Postharvest treatments, such as application of reduced-risk fungicide, biological agents and natural products, heat treatment and edible coating formulations alone or in combination, can be successfully applied in a range of commodities in order to prevent decay. Until a long time ago, synthetic fungicides as benzimidazole, captan, diphenylalamin, dithane, and dicarboximide [9], have been used for handling of destructive molds on postharvest fruit at the farm level. However, long-term use of fungicide can be accumulated in the human body and imply to cause serious diseases [11, 12]. Therefore, the mode of action of antagonistic yeast in postharvest fruit disease control could be an important tool in postharvest biocontrol strategies, thus providing important guidance for their future application. In addition, mixtures of low-risk fungicides with biological agents should be carried out to identify the best postharvest treatments with the lowest environmental impact and the greatest consumer safety.

Microorganisms such as yeasts can be used as biological agents since it can produce compounds that are beneficial to humans and animals [13, 14]. Yeast has the ability to fight against destructive microorganisms [15, 16]. The ability of epiphytic yeast antagonism is shown through the ability of yeast to inhibit the growth of other microorganisms around it [17-19]. This can be further used as biocontrol agents.

Some epiphytic yeasts have been reported to inhibit mold growth [20, 21]. It has been reported that *Metschnikowia pulcherrima* BIO126 and *M. pulcherrima* GS37 isolated from apple surface can inhibit the growth of pathogenic molds, *Alternaria* sp., in apples [2]. In other research, it is found that glutinised *Rhodotorula* can inhibit the growth of pathogenic molds in apples after harvest period of *P. expansum*, shown by the results of *in vivo* and *in vitro* testing [22, 23]. *R. mucilaginosa* could inhibit the growth of *P. expansum* and *B. cinerea* in postharvest apples. The results of the interaction were demonstrated through the reduction of *B. cinerea* spore germination and reduction of colonies, which was greater than the control [24, 25]. *Candida oleophila* epiphytes isolated from the surface of tomatoes has also shown its ability to inhibit the growth of *Penicillium expansum* and *Botrytis cinerea* in postharvest kiwi fruit through antioxidant mechanisms which can be considered the presence of antioxidant gene expression [26].

Plants are substrates that can be overgrown by yeast [27, 28]. Bintaro (*Cerbera manghas* L.) is one of the plant types that can be overgrown by yeast [20]. The chemical content of leaves, flowers and fruit in Bintaro plants, including saponin, polyphenols, tannins, steroids, and flavonoids [29-31], are widely used in the pharmaceutical industry as ingredients of the medicine. The presence of these chemical contents might be the result of the existence of microorganisms. The identification of molds and yeasts was performed based on the data sequences of ITS region which have high variability between species so that they can be used to identify yeast at the species level [27, 32]. The ability of epiphytic yeast to inhibit the growth of destructive molds on fruit can be done by a dual culture method [20, 33]. Therefore, this study was aimed to identify and screen the inhibition activity of yeast isolated from *Cerbera manghas* L. against the growth of destructive molds in apples originating from Malang, Indonesia. Also, this research was expected to find the potential yeast able to act as biocontrol agents to apply in postharvest fruit production, especially in apples.

2. MATERIALS AND METHODS

2.1. Yeasts Collection and Sampling

A total of 19 yeast isolates were collected from the Universitas Negeri Jakarta Culture Collection. It isolated from Bintaro plants. Yeast was cultivated on two media, Peptone Dextrose Yeast Extract (10 g/L yeast extract, 20 g/L peptone and 20 g/L dextrose), and Nutrient Yeast Dextrose Broth (8 g/L nutrient broth, 5 g/L yeast extract and 10 g/L glucose) on a rotary shaker at 180 rpm for 24h at 28°C. The cell suspension was centrifuged at 4000°g for 10 min at 4°C, followed by washing with distilled water to remove the growth medium. Cultivated yeasts were then suspended in the concentration of 1 to 5 × 10^8 cells/mL.

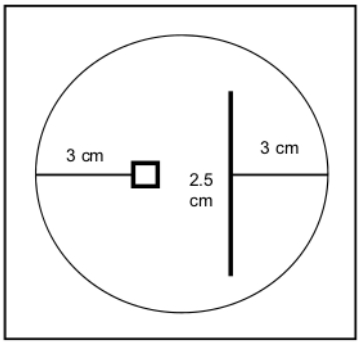
Fresh apples are obtained from the Jatinegara market, with the same size and maturity. The untreated apple fruits were washed, superficially disinfected with 0.2% (v / v) sodium hypochlorite for 3 min and reduced distilled water to eliminate the sodium hypochlorite. The fruits were then wounded to two points at the median region with sterile needles. The destructive mold was obtained from damaged apples and was followed by pathogenicity testing [34]. The two pathogens found were then coded by A1 and A17 isolates and maintained on Potato Dextrose Agar (PDA; 200 g/L extract of boiled potatoes, 20 g/L glucose and g/L agar) at 4°. Spores of mold isolates with the code A1 and A17 were obtained from 6-day-old cultures on PDA at 25°C and suspended in sterile distilled water containing 0.1 g/kg Tween-80. The concentration of spore suspension was adjusted to the concentration of 1×10^7 spores/mL.

2.2. Identification of Molds Isolates by Amplification of the ITS Regions of the rDNA

Two molds isolates (A1 and A17) were identified based on their genetic material. DNA was extracted from the yeasts using DNA using the Genetic Plant Gneaid kit. ITS (Internal Transcribed Spacer) rDNA amplification was performed using the following primers: forward- ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and reverse-ITS4 (5'TCCTCCGCTTATTGATATGC-3') according to White *et al*. PCR cycling conditions for yeasts consisted of an initial denaturation step at 95°C for 3 min; 33 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min; and a final extension at 68°C for 10 min.

2.3. *In Vitro* Screening of Yeast Isolates for Antifungal Activity

The antagonicity tests were performed to all 19 yeast isolates obtained from Bintaro leaves. The antagonism testing method was using a dual culture method based on Sibounnavong *et al*. [35] and Sukmawati *et al*. [20] with modification. Tests were carried out in the Yeast Malt Agar (YMA) medium. For each test, one mold isolate and one yeast isolate were inoculated on the same petri dish where the distance between the two was 2.5 cm (Fig. **1**). A total of 20 µl of yeast cell suspensions with densities of 1 to 5 × 10^8 cells/mL were inoculated on the side of one pathogenic mold (A1 and A17) with spore densities of 1 × 10^7 spores/mL inoculated on the other side. Incubation was carried out for 5 days at 28°C. Observations were made on the fifth day by measuring the width of the inhibition zone between yeast and destructive molds using digital calipers. A completely randomized design with four replications for each of the 3 trials was used. The data were subjected to analysis of variance (ANOVA). The means were tested by Tukey's 5% probability.



Notes: ◘ Mold pathogen

I Yeast isolates from apples (with 6 cm long)

Fig. (1). Yeast and mold antagonism test using YMA dual culture medium, incubated at 28°C.

2.4. Calculation of Yeast Cell Growth Curves

Yeast cell growth curves were constructed based on Tang & Amon [36] with modification. The results of the measurement of the number and duration of the yeast log as a reference for the duration of yeast fermentation will be used for biocontrol.

2.5. Identification of Yeast Isolates by Amplification of the ITS Regions of the rDNA

Three yeast isolates (T1, T3 and T4) were identified based on their genetic material. DNA was extracted from the yeast using the Genetic Plant Gneaid kit. DNA was amplified based on the ITS (Internal Transcribed Spacer) rDNA region using the following primers: forward- ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and reverse-ITS4 (5'TCCTCCGCTTATTGATATGC-3'), according to White *et al*. [39]. PCR cycling conditions for yeasts consisted of an initial denaturation step at 95°C for 2 min; 33 cycles of denaturation at 95°C for 15 sec, annealing at 58°C for 30 sec and extension at 68°C for 2 min; and a final extension at 68°C for 10 min. The PCR product was purified with first base service. The sequences were aligned and compared with the NCBI database by the Internet using the Basic Local Alignment Search Tool [40].

2.6. *In Vivo* Antagonistic Activity Assays

In vivo method is carried out using the cut method based on Mahunu *et al*. [37] with modifications. Isolates of destructive molds and yeast were suspended under 7 treatments: 1) fresh apples without washing or surface sterilization; 2) apples washed with tap water; 3) apples carried out surface sterilization; 4) apples soaked with yeast suspension; 5) apples inoculated with mold; 6) apples soaked with yeast suspension then inoculated with mold; 7) apples are soaked with dithane M-45 0.3% then inoculated with mold. The prepared apples are soaked with yeast cell densities of 1 to 5 × 10^8 cells / mL, followed by incubation for 24 hours. Soaked apples with yeast suspension are then inoculated with 20 µl of pathogenic fungus spores 1 × 10^7 spores/ mL spore density. The treated apple was placed in a plastic tube and covered with clear plastic. The four corners are like wet cotton pads to maintain moisture followed by incubation at 27-28°C for 7 days. Observations were made every day until the seventh day of incubation after inoculation to determine scoring on a scale of 0, 2, 4, 6, and 8 [38]. The identification of plant resilience is done by calculating the percentage of Disease incidence (DI) and Disease Severity [38].

3. RESULTS

3.1. Identification of Mold Isolates by Amplification of the ITS Regions of the rDNA

Sequences of A1 and A17 isolates were then aligned with various sequences of *Aspergillus* species in the NCBI DNA GenBank database. Based on the phylogenetic tree (Fig. **2**), isolates A1 and A17 are in different clades. Isolate A1 was also in a monophyletic clade in the nigri section with *A. heteromorphus* CBS 117.55 and *A. brasiliensis* ATCC MYA-4553 with a bootstrap value of 88%. Meanwhile, isolate A17 was in a monophyletic clade in section flavi with *A. flavus* ATCC 16883, *A. lanosus* NRRL 3648 and *A. oryzae* NRRL 447 with a bootstrap value of 90%.

3.2. *In Vitro* Screening of the Yeast Isolates for Antifungal Activity

The antagonism test showed that yeast isolates from Bintaro leave on the MEA medium could inhibit the growth of apple-damaging molds. This is indicated by the presence of clear zones between mold colonies and yeasts. The clear zone is a zone of inhibition of mold colonies mycelium growth, as seen in Fig. (**3**).

The results of the two-way ANOVA analysis showed that there was an effect of giving yeast isolates to the inhibition of growth of test fungi, which is the Sig. 0.00 <α (0.05%) (Table **1**).

Based on the Duncan test at the level of 5%, it can be seen that yeast isolates with the code of T1 and T4 have no significant differences in inhibition of growth of *A. brasiliensis*

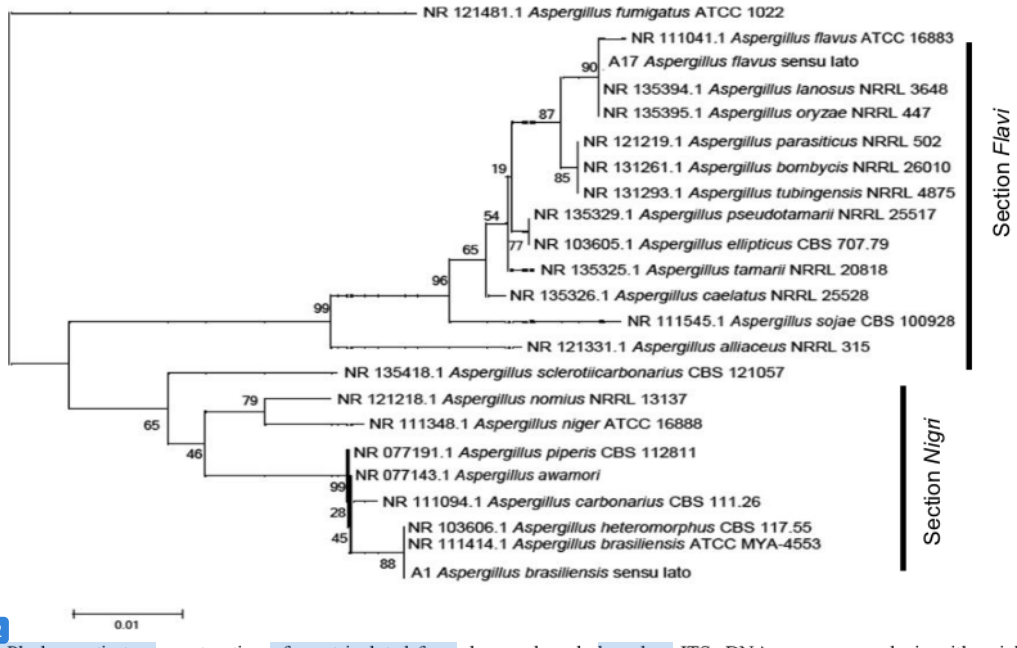


Fig. (2). Phylogenetic tree construction of yeast isolated from damaged apple based on ITS rDNA sequence analysis with neighbor joining method 1000 times bootstrap, MEGA5. *A. fumigatus* ATCC 1022 is used as an outgroup.

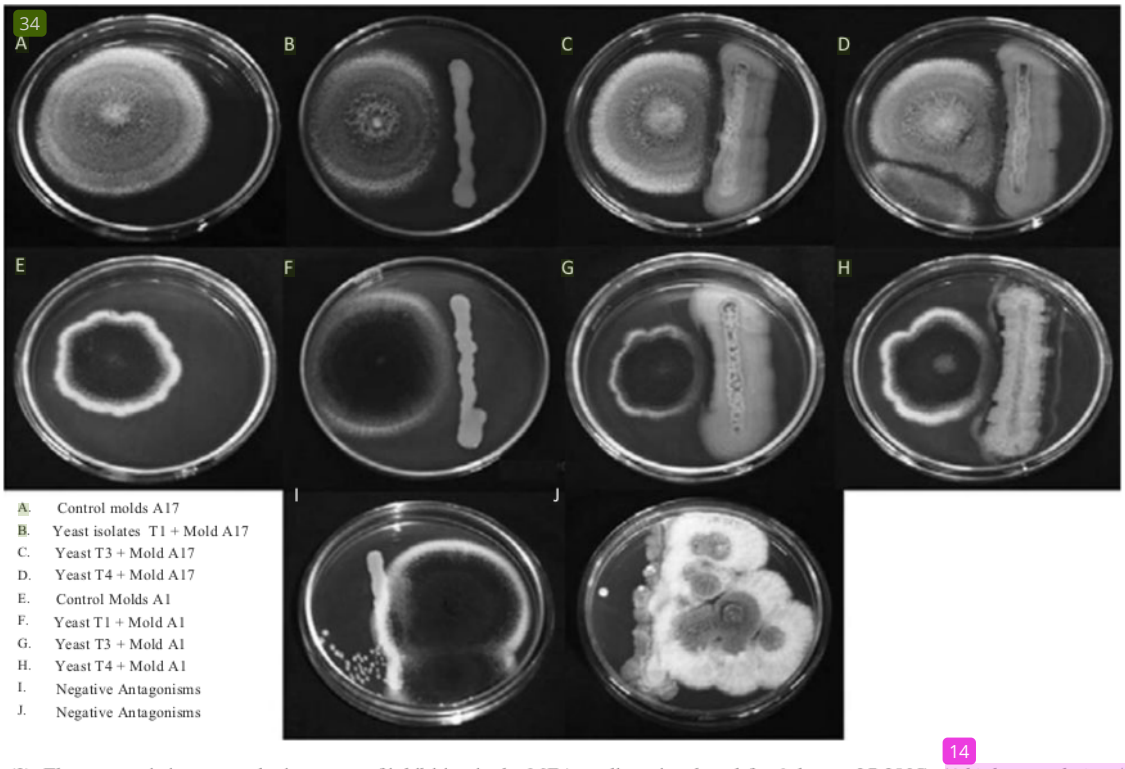


Fig. (3). The antagonistic test results in a zone of inhibition in the MEA medium, incubated for 6 days at 27-28°C. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 1. The width of the inhibitory zone formed in the antagonistic test of 19 yeasts originating from Bintaro leaves against two apple decay molds.

A. brasiliensis Sensu Lato		*A. flavus* Sensu Lato	
Yeasts	**Inhibition zone (mm) ±SE**		**Yeasts**
T1	1,06 ±0,26[a]	0,67 ±0,35[a]	T1
T2	0,00	0,00	T2
T3	1,16 ± 0,09[b]	1,52 ±0,08[b]	T3
T4	1,00 ±0.16[a]	0,70 ±0,13[a]	T4
T5	0,00	0,00	T5
T6	0,00	0,00	T6
T7	0,00	0,00	T7
T8	0,00	0,00	T8
T9	0,00	0,00	T9
T10	0,00	0,00	T10
T11	0,00	0,00	T11
T12	0,00	0,00	T12
T13	0,00	0,00	T13
T14	0,00	0,00	T14
T15	0,00	0,00	T15
T16	0,00	0,00	T16
T17	0,00	0,00	T17
T18	0,00	0,00	T18
T19	0,00	0,00	T19

Table 2. The width of the inhibitory zone formed in the test of yeast antagonists from bintaro leaves on two apple-damaging yeasts on PDA medium, incubated in 6 days with a temperature of 27-28°C.

Yeasts	**Inhibition Zone (mm) (Mean[D] ± SE)**
	Molds
T1	0,87[a] ± 0,20
T3	1,33[b] ± 0,18
T4	0,86[a] ± 0,13

Note: Numbers followed by the same letters are not significantly different at α = 0.05 Duncan test. Results that have a 0 zone value (negative) are not displayed.

sensu lato and *A. flavus* sensu lato. This is because the width of the inhibition zone formed against *A. brasiliensis* sensu lato (1.16 ± 0.09c) and *A. flavus* sensu lato (1.52 ± 0.08c) is the largest inhibition zone width (Table **1**). Yeast isolate with the code of T3 has a significant difference compared to the other two yeasts in inhibiting the growth of test fungi. Isolate T3 is the most potential yeast in inhibiting the growth of *A. brasiliensis* sensu lato and *A. flavus* sensu lato (Table **2**).

3.3. Identification of Yeast Isolates by Amplification of the ITS Regions of the rDNA

Sequences of yeast isolate T1, T3 and T4 were aligned with various sequences of *A. namibiae, A. thailandense, A. subglaciale, K. bupleuri, R. glutinis, R. graminis, R. araucariae*, and *R. kratochvilovae* which were downloaded from the DNA database NCBI GeneBank. The search results for ITS rDNA sequence homology using BLAST program showed that isolate T1 was identified as *R. mucilaginosa* with ITS sequence homology of 94% with the closest species, *R. mucilaginosa* CBS 316. Compared to the three sequences, namely *A. pullulans* (0.15%), *K. lines* (0.31%) and *A. namibiae* (0.15%), T3 and T4 isolates showed differences in nucleotide bases (Fig. **4**). Isolate T3 and T4 are later identified as *Aureobasidium* sp. nov.

Phylogenetic trees were made using MEGA 5 software with the Joining Neighboring (NJ) method. Phylogenetic

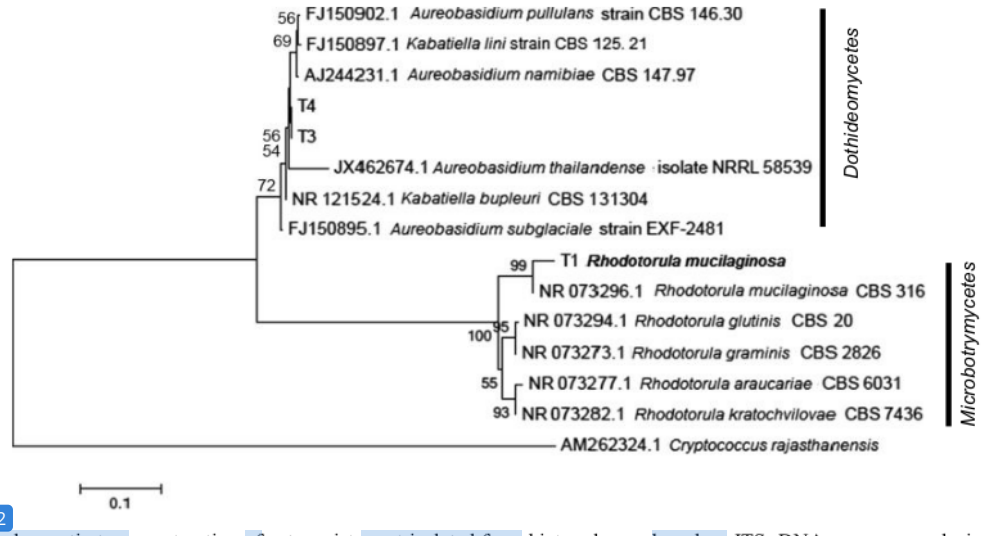
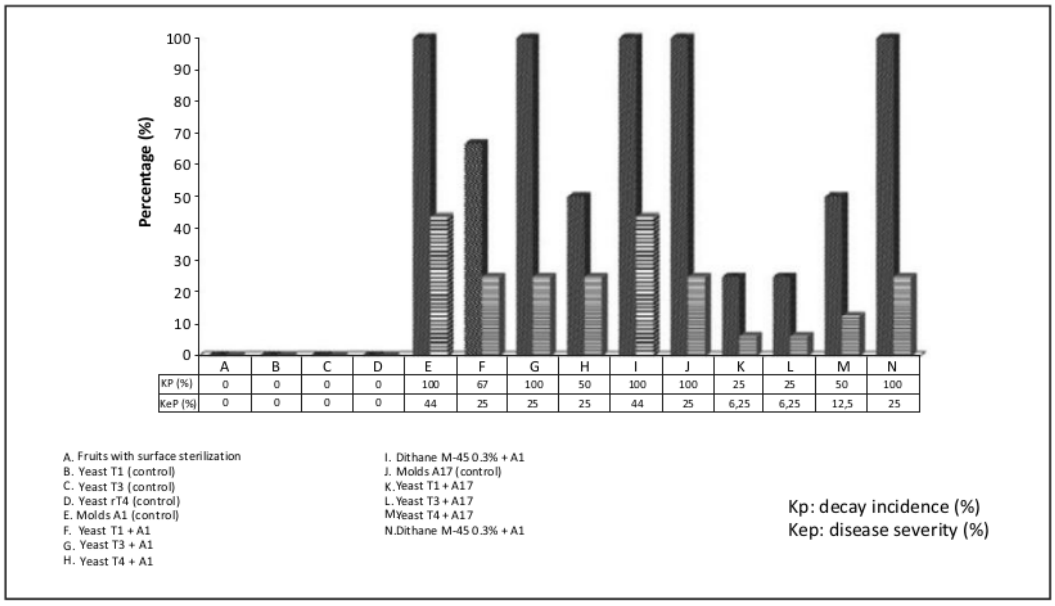


Fig. (4). Phylogenetic tree construction of antagonist yeast isolated from bintaro leaves based on ITS rDNA sequence analysis with Neighbor-Joining method 1000 times bootstrap, MEGA5. *Cryptococcus rajasthanensis* is used as an outgroup.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
KP (%)	0	0	0	0	100	67	100	50	100	100	25	25	50	100
KeP (%)	0	0	0	0	44	25	25	25	44	25	6,25	6,25	12,5	25

Fig. (5). Percentage of decay incidence and disease severity in antagonistic biocontrol T1, T3, and T4 tests against *A. brasiliensis* sensu lato and *A. flavus* sensu lato, incubated for 7 days at 27-28°C.

trees are used to determine kinship relationships between species sampled with various other species. Molecular phylogenetic combines molecular biology techniques with statistics to reconstruct phylogenetic relationships [41].

3.4. *In Vivo* Antagonistic Activity Assays in Damaged Apples

Based on *in vitro* test, isolate T1, T3, and T4 have the potential ability to act as biocontrol agents for *A. brasiliensis* sensu lato and *A. flavus* sensu lato. Yeast isolates showed different abilities from one another. Tests of antagonistic yeast biocontrol on destructive mold showed the percentage of rotten apples that varied with incubation of 6 days. Isolate T4 (decay incidence 50%; disease severity 25%) have the ability as superior biocontrol agents against the growth of *A. brasiliensis* sensu lato compared to isolate T1 (decay incidence 67%; disease severity 25%) and T3 (decay incidence 100%; disease severity 25%). The ability of yeast isolates T1, T3, and T4 to reduce the growth of *A. brasiliensis* sensu lato was better than that of Dithane M-45 synthetic fungicide 0.3% (decay incidence 100%; disease severity 44%) (Figs. **5** and **6**).

Yeast isolates of T1, T3, and T4 can reduce the growth of *A. flavus* sensu lato, thereby reducing apple rot. Yeast iso-

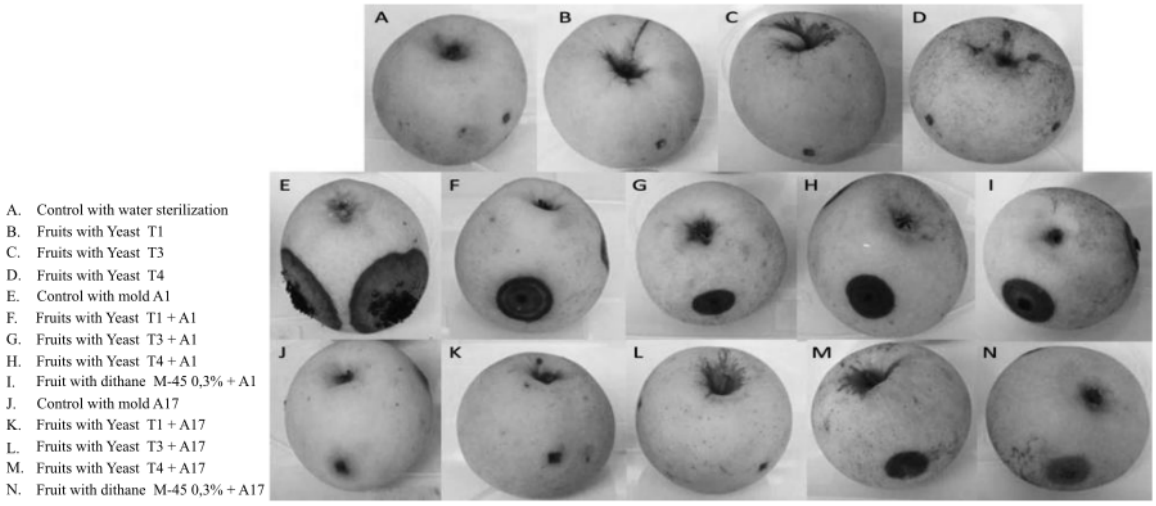


Fig. (6). Biocontrol test of yeast isolated from Bintaro leaves against destructive molds with incubation of 7 days at 27-28°C. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

lates T1 and T3 (decay incidence 25%; disease severity 6.25%) have the ability as superior biocontrol agents against the growth of *A. flavus* sensu lato compared to T4 yeast isolates (decay incidence 50%; disease severity 12.5%). The ability of T1 and yeast T3 in reducing the growth of *A. flavus* sensu lato in apple rot is better than that of Dithane M-45 synthetic fungicide 0.3% (decay incidence 100%; disease severity 25%).

4. DISCUSSION

The results of this study showed that 19 yeast isolates tested were obtained from 3 isolates (T1, T3 and T4) of epiphytic yeast from the Bintaro plant which has antagonistic ability to inhibit the growth of mold pathogens that caused damage to postharvest apples (*A. brasiliensis* sensu lato and *A. flavus* sensu lato). It is convergence with other works conducted by other researchers [8, 25, 42] who have reported the successful biological fungal treatment using yeast agents.

The results of the 2-way ANOVA analysis showed that there was an effect of the presence of yeast isolates to inhibit the growth of destructive molds shown by Sig. 0.00 <α (0.05%). This is in line with the statement of Golubev [18] that the ability of yeast antagonism will be more increased against microorganisms from different habitats since they are considered as new competitors that must be defeated to be able to dominate the available space and nutrients. The competition between yeast and mold can be demonstrated by the rise of yeast growth in the medium. Yeast cells have the ability to absorb nutrients in the medium more than mold cells. Molds will get a lack of nutrients to grow at the same medium of yeast growth so that the mycelium formed became less. Mechanism of space and nutrient competition occurs when yeasts try to obtain limited space and nutrients when grown with pathogens [43]. The growth activity of mold colonies is disrupted due to lack of nutrients and space to grow [44-56].

The search results for ITS rDNA sequence homology using the BLAST program showed that *A. brasiliensis* sensu lato is located in a monophyletic clade in the nigiri section with *A. brasiliensis* ATCC MYA-4553 with an ITS sequence homology of 98% with the closest species, *A. brasiliensis* ATCC MYA-4553. This mold is known as *Aspergillus brasiliensis* sp. nov., which can be distinguished from other black aspergilli based on intergenic transcribed region, beta-tubulin and calmodulin gene sequences. It is an aerobic and produced naphtho-gamma-pyrones, tensidol A and B and pyrophen in common with *Aspergillus niger* and *Aspergillus tubingensis*. This species was most closely related to *A. niger*, and was isolated from soil from Brazil, Australia, USA and The Netherlands, and from grape berries from Portugal [53]. In other words, A1 *A. brasiliensis* sensu lato is possible to cause damage in postharvest fruits since it is also found in damaged grape berries.

A. flavus sensu lato is closely related to *A. flavus* ATCC 16883, which is known as aflatoxin-producing mold. This isolate was found on stored products [53] and Argentinean peanuts [54]. *A. flavus* sensu lato has a sequence homology of 99% with *A. flavus* ATCC 16883. From molecular identification result, it can be seen that killer yeast can be found on its natural habitat since plants are good resources for the yeast to grow [53]. The inhibition activity occurs when there is a limitation in nutrients.

The antagonistic test results showed that three antagonistic isolates (T1, T3, T4) have the ability to inhibit the growth of two destructive molds, while the other isolates did not represent inhibitory zones. Isolates T3 is the most potential pathogen-inhibiting yeast for isolate *A. brasiliensis* sensu lato and *A. flavus* sensu lato. The width of the inhibitory

zone by T3 yeast isolates is 1.33 mm, as seen in Table **1**. In this study, it is assumed that the greater number of inhibition zone value formed, the greater the ability of yeast to inhibit mold growth. The inhibition of mycelium growth in mold colonies is predicted due to competition for nutrition and space. The competition between microorganisms for essential environmental factors, such as those of the fundamental mechanisms of biological control [7, 24, 50].

Yeast isolates of T1, T3, and T4 were aligned with various sequences of *A. namibiae, A. thailandense, A. subglaciale, K. bupleuri, R. glutinis, R. graminis, R. araucariae,* and *R. kratochvilovae* which are downloaded from the DNA database NCBI GenBank. Isolate T1 was identified as *Rhodotorula mucilaginosa,* which is located in a monophyletic clade together with *R. mucilaginosa* CBS 316 with a 99% bootstrap value. In recent years, several yeast species (mainly of the genera of *Rhodotorula* and *Crytococcus*) have been claimed to be useful as for the biological control of postharvest disease on storage fruits and vegetables due to their antagonistic activities against common plant pathogens. Among the *Rhodotorula* species reported as potential biocontrol agents, *R. mucilaginosa*, *R. glutinis*, and *R. minuta* are included and have been tested with promising results. Some researchers have carried out antagonistic testing of yeast on apple decay such as yeast antagonism on molds of *Colletotrichum* sp. from strawberries, chili and beans. Yeast *Rhodotorula* sp. is able to inhibit the growth of *Colletotrichum* sp. from chili and strawberry fruits by 40% and 55% with incubation for 10 days [16, 33, 34, 51]. Yeast *Metschnikowia* sp. can inhibit the growth of *Colletotrichum* sp. from chickpea by 35% with incubation for 10 days. *Pichia guilliermomdii* and *R. mucilaginosa* also showed its ability to inhibit the growth of blue mold on apple damage, *Penicillium expansum,* tested using the dual culture method. Antagonist testing using PDA medium with incubation for 18 days at 25°C. The average percentage of inhibition zones formed by *Pich. guilliermomdii* at 57.62% and *R. mucilaginosa* at 34.51% [52].

The broad characteristics occurrence in natural and artificial environments of *Rhodotorula* species, especially of *R. mucilaginosa,* is most certainly a result of their physiological and metabolic plasticity. Such characteristic is also responsible for their frequent appearance in food and beverages. Food and beverages can be a significant source of *Rhodotorula* yeasts, thus posing an additional and probably underestimated risk for susceptible patients.

Based on phylogenetic construction, isolate T3 and T4 are predicted as new species since they formed an independent lineage from their closest sequences, *A. Pullulans, K. line* and A. *namibiae* with a bootstrap value of 56%. This showed that isolate T3 and T4 have a homology level that is not close to the three sequences. Isolate T3 and T4 have unique gene sequences, which do not align with any sequences in the databases and showed a difference in nucleotide bases compared to the three sequences, which are *A. pullulans* (0.15%), *K. lines* (0.31%) and *A. namibiae* (0.15%). T1 isolate sequences have a difference of 0.94% with *A. pullulans* sequences downloaded from NCBI. *Aureobasidium pullulans* is a ubiquitous black, yeast-like fungus that can be found in a wide range of environments. It is well known as a naturally occurring epiphyte or endophyte of many plant species without causing any symptoms of the disease.

CONCLUSION

This study showed that yeasts isolated from Bintaro leaves have antagonism and biocontrol activity against destructive molds isolated from Malang apples. Isolate of T1 which is identified as *Rhodotorula mucilaginosa* and isolates of T3 and T4 which were identified as *Aureobasidium* sp. nov., were found to have the ability to grow against *A. brasilensis* sensu lato *and A. flavus* sensu lato with an inhibition zone of 0,87 ± 0,20, 1,33 ± 0,18, and 0,86 ± 0,13, respectively. The inhibition process in the antagonistic test to the three yeast strains showed inhibition in the sporulation process and the formation of the distance of the inhibition zone between yeast and mold mycelium growth. In this study, it is found that epiphytic yeast isolates from Bintaro leaves have the ability to reduce disease severity and disease incidence by almost 100% of apples compared to Dithane M-45 synthetic fungicide. Yeast application as a biocontrol agent can be used as an alternative in substituting pesticide use, which is very dangerous for both humans and the environment. In future studies, we will further explore how yeast isolates interact with pathogenic molds.

CURRENT & FUTURE DEVELOPMENTS

Further studies will be conducted to determine the environmental factors that affect yeast viability, such as pH, temperature, carbon sources that are expected to increase the role of yeast as a postharvest biocontrol agent in apples. This study contributes to biocontrol research that epiphytic yeast obtained from Bintaro plants can be used as a biocontrol agent in controlling pathogenic fungi in postharvest fruit.

AUTHOR CONTRIBUTIONS

DS, IH, MN conceived and designed the experiments; AS and RI performed the experiments; DS, DJD, IH analyzed the data; DS, SNA, THK, NIR, HEE, AEH wrote the paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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