INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0063/2024/32–3–219–226
DOI: 10.17957/IJAB/15.2195
http://www.fspublishers.org

Full Length Article

Evaluation of Phyllosphere and Endophytic Yeast as Biofertilizer and Growth Promoter of Red Cherry Tomato

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Received 01 February 2024; Accepted 16 May 2024; Published 26 July 2024

Abstract

Among Indonesia's cultivated crops, tomatoes (including the smaller red cherry types) are popular. However, the production of red cherry tomatoes has failed to meet the demand, largely due to the significant impact of the fungal pathogen *Fusarium oxysporum lycopersici*, which has led to a decrease in tomato productivity by up to 40%. Addressing this issue is crucial for achieving sustainable red cherry tomato production. The use of synthetic fertilizers poses environmental concerns, including soil, water, and air pollution. Therefore, exploring yeast as a plant growth promoter in biofertilizers presents a promising alternative or partial substitution fertilizer opportunity. In this study, we characterized 63 yeast isolates based on their ability for phosphorus solubilization, ammonia production, hydrogen cyanide (HCN) production, indole-3-acetic acid (IAA) production, and antifungal activities against *F. oxysporum*, *Cercospora beticola* and *Alternaria porri*. Among the isolates, four showed the most promising characteristics and were further identified through molecular techniques. *In vivo* experiments were conducted utilizing red cherry tomatoes to evaluate the effectiveness of these isolates. The phosphate solubilization test revealed that 11 yeasts exhibited high solubilization index (SI) activity (SI > 2). None of the isolates tested positive for ammonia or HCN production, but two showed a positive result for the IAA production test. Antifungal activity assays demonstrated that 63 yeasts could inhibit *F. oxysporum* and *C. beticola*, while 62 yeasts inhibited *Al. porri*. Based on sequencing results of the ITS2 region, the four identified isolates were assigned as follows: C4 as *Cyberlindnera fabianii*, C7 as *Cutaneotrichosporon mucoides*, C10 as *Yarrowia lipolytica* and K2 as *Aureobasidium melanogenum*. *In vivo* experiments confirmed that all four isolates effectively reduced the presence of *F. oxysporum*. Notably, *C. mucoides* exhibited the strongest inhibition ability, as indicated by a disease incidence rate of 51.10%.

Keywords: Biofertilizer; Fungal pathogen; Cherry tomato; Yeast; Hydrogen cyanide

Introduction

Tomatoes (*Solanum lycopersicum* L.) are widely consumed and cultivated globally, including in Indonesia. Indonesia ranks as the third-largest tomato producer in the Asia Pacific region, following China and India. Tomato production in Indonesia varies significantly across its regions. Statistics stated that West Java is the leading producer of tomatoes in Indonesia. As per 2023, West Java produced 283,587 tons (Rafani and Dabukke 2024). Red cherry tomato is a rich source of bioactive metabolites such as vitamin A, vitamin C, protein, lycopene, carotene, lutein, rutin and glutathione (Yunita and Isnaeni 2020; Yang *et al*. 2023). Red cherry tomatoes also contain minerals such as potassium (K) and magnesium (Mg) (Yang *et al*. 2023) and are high in fructose and glucose concentration (Borjska *et al*. 2020).

Red cherry tomatoes, in particular, have gained popularity among Indonesians, leading to an annual increase in demand. Characterized by their sweetness taste and vibrant red coloration, cherry tomatoes offer a conveniently compact form ideal for immediate consumption into various culinary applications. In addition, the reduced seed content further enhances their consumer appeal (Guilherme *et al*. 2014). In 2017, cherry tomato consumption in Indonesia reached 962,849 tons per annum but domestic production somewhat reduced, necessitating imports as a solution (Nawaal *et al*. 2022). However, the productivity of tomatoes in Indonesia is known to be unstable, largely attributed to

To cite this paper: Magdalena S, VE Rarisavitri, Yogiara (2024). Evaluation of phyllosphere and endophytic yeast as biofertilizer and growth promoter of red cherry tomato. *Intl J Agric Biol* 32:219–226

diseases, including fungal infections (Anisa *et al*. 2022). Globally, *Fusarium oxysporum lycopersici* has caused a decline in tomato production by up to 40% (Hassan 2020). Additionally, factors such as poor soil fertility, limited cultivation areas, and pest infestations have contributed to the low productivity of cherry tomatoes (Mardaus and Yusuf 2019). Consequently, the implementation of growth promoters and biofertilizers is crucial to enhance cherry tomato production.

The utilization of synthetic fertilizers often leads to environmental issues such as soil, water, and air pollution due to the presence of heavy metals (Ajmal *et al*. 2018). The use of fertilizers containing nitrogen can contribute to air pollution. When fertilizers are applied in excess, they release nitrogen oxides into the atmosphere. These oxides can then react with other elements to form harmful pollutants, including water vapor, carbon dioxide, methane, and hydrogen sulfide (H_2S). Additionally, some fertilizer production processes may involve chlorofluorocarbons (CFCs), such as halon gases, which further contribute to air pollution concerns (Ajmal *et al.* 2018).

In recent decades, the use of biofertilizers has become increasingly popular due to their advantages over synthetic fertilizers. Biofertilizers, which consist of substances containing live microorganisms, have been recognized for their ability to enhance plant growth by improving nutrient availability when applied to plants (Sharma *et al*. 2022; Dawood *et al*. 2023). By enriching the soil with beneficial microorganisms, biofertilizers have been successfully employed to increase crop productivity and improve soil health. A key advantage of biofertilizers is their ability to mitigate the negative impacts of chemical fertilizers, such as releasing heavy metals that contribute to soil, water, and air pollution (Ajmal *et al*. 2018).

The exploration of plant growth-promoting microorganisms has emerged as a promising opportunity. Studying these microorganisms holds great potential for promoting sustainable crop production. Among these microorganisms, yeast can enhance plant growth through the provision of nitrogen and phosphorus, organic acids, and phytohormones (Lonhienne *et al*. 2014).

Within the soil environment, yeasts play a crucial role in maintaining ecological balance by facilitating nutrient recycling, decomposition processes, and supplying nutrients through their biological activity, thereby enhancing soil health (Al-Atrash *et al*. 2021). Yeasts are known to form associations with their hosts, such as symbiotic interactions. Among them, plant-growth promoting yeasts (PGPY) have the ability to colonize various parts of plants, including the endophyte and phyllosphere and confer beneficial effects. These yeasts contribute to nutrient availability and modulate the production of phytohormones, making them a valuable group in agriculture (Nimsi *et al*. 2023). Consequently, the potential of yeasts in the field of agriculture has garnered significant research interest in recent years. Furthermore, endophytic yeast, which resides within a plant's tissues, has been found to stimulate plant growth (Hernández-Fernández *et al*. 2021). *Candida*, for instance, has garnered attention for its biofertilizer potential due to its ability to solubilize phosphate and produce indole-3-acetic acid (IAA) (Hernández-Fernández *et al*. 2021).

Sixty-three yeast isolates were obtained from various species of ornamental plants by Indriana (2019). The objective of this study was to characterize these yeast isolates through multiple tests, including phosphorous solubilization, ammonia production, hydrogen cyanide (HCN) production, IAA production, antifungal activities against *F. oxysporum*, *Al. porri* and *C. beticola*; molecular identification of the Inter Transcribed Sequence (ITS) 2 region; and *In vivo* experiment on red cherry tomatoes.

Materials and Methods

Culture preparation

The yeast used in this study was isolated by Indriana (2019) from the phyllosphere and ornamental plants listed in Table 1. The yeast was cultured on Yeast Extract Peptone Dextrose (YEPD) agar medium (Cold Spring Harbor Protocols 2017), which consisted of 1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose and 2% (w/v) agar. Additionally, the medium was supplemented with chloramphenicol (250 g/mL). The YEPD agar plates were then incubated at 30°C for a period of two days.

Phosphorus solubilization test

The phosphorus solubilization test was conducted following the methodology outlined by Gizaw *et al.* (2017). Yeast isolates were spotted onto sterile solid Pikovskaya media, which contained 5 g L^{-1} of calcium phosphate as the phosphate source. The plates were then incubated at 30°C for 5 days. Each treatment was replicated three times. Phosphorus-solubilizing isolates were identified by the formation of clear zones around the yeast colonies, indicating phosphate dissolution. The extent of solubilization was measured using the solubilization index (SI). An SI value greater than 1 indicated that the isolates possessed phosphate solubilization activity, while an SI value greater than 2 indicated high phosphate solubilization activity (Kumla *et al.* 2020).

$$\text{Solubilization Index (SI)} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

Ammonia production

The ammonia production test was conducted using urea broth following the methodology described by Galeano *et al.* (2021). Yeast isolates were cultured in YEPD broth medium and incubated at 30°C in shaking incubator at 150 rpm for 5 days. After centrifugation at 3000 × g for 5 min, one milliliter of supernatant was mixed with 50 μL of

Nessler's reagent (composed of 10% Mercury (II) Iodide (HgI_2), 7% Potassium Iodide (KI) and 50% of a 32% NaOH solution) in test tubes. There were three replications for each isolate. Urease-producing isolates were identified by the development of a brownish color.

Hydrogen cyanide (HCN) production

The HCN production test was conducted following the procedure outlined by Bright *et al.* (2022). Yeast isolates were streaked onto Kings B medium, which consisted of 20 g L^{-1} protease peptone, 1.5 g L^{-1} dipotassium hydrogen phosphate, 1.5 g L^{-1} magnesium sulfate heptahydrate, 15 mL L^{-1} glycerol and 15 g L^{-1} agar. The medium was supplemented with 4 g L^{-1} glycine. Filter paper was soaked in a solution of 2% sodium carbonate in 0.5% picric acid and placed inside the lid of each plate. The plates were sealed airtight with parafilm paper and incubated at 30°C for 4 days. Three replications were performed for each isolate. The HCN production was identified by the change in color of the filter paper from deep yellow to reddish brown.

Indole-3-acetic acid (IAA) production

The IAA production test was conducted following the method described by Sun *et al.* (2014). Yeast isolates were cultured in test tubes containing YEPD broth medium and incubated on a shaker at 30°C and 150 rpm for 5 days. After centrifugation of 1 mL of the cell culture at 3000 × g for 5 min, 0.5 mL of the supernatant was mixed with 0.5 mL of Salkowski reagent, which consisted of 2 mL of 0.5 *M* iron (III) chloride and 98 mL of 35% perchloric acid. Three replications were performed for each isolate. The production of IAA was indicated by a color change to red.

Antifungal activities

The antifungal activity test was conducted following the methodology described by Elkahoui *et al.* (2012) with slight modifications. *F. oxysporum* InaCC F219, *C. beticola* InaCC F194 and *Al. porri* InaCC F195, obtained from Indonesian Culture Collection (https://inacc.brin.go.id/), were utilized as fungal pathogens. The fungi were cultured on Potato Dextrose Agar (PDA) and incubated at 30°C for 5 days to allow hyphal formation. Agar plugs with a diameter of 7 mm were prepared using a cork borer. Yeast isolates were streaked on Malt Extract Agar (MEA) at 1.5 cm from the center and incubated at 30°C for 2 days. The previously prepared agar plug was placed in the center of the plate. Two replications were performed for each isolate. The degree of inhibition was calculated using a formula (Elkahoui *et al.* 2012):

$$\% \text{ Inhibition} = \frac{D1 - D2}{D1} \times 100\%$$

D1 = Diameter of fungi growth on negative control (mm)
D2 = Diameter of fungi growth on each yeast isolate (mm).

Molecular identification of potential yeast isolates

The molecular identification of yeasts was conducted following the modified method described by Beeck *et al.* (2014). Yeast isolates were cultured on YEPD broth medium for 24 h and DNA was extracted from each isolate using the ZymoBIOMICS DNA miniprep kit (Zymo Research, USA). The concentration of DNA was measured at 260 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Molecular identification was performed through PCR amplification and sequencing of the ITS2 region. ITS2 amplification was achieved using primers GCATCGATGAAGAACGCAGC and TCCTCCGCTTATTGATATGC (Beeck *et al.* 2014).

For PCR, the reaction mixture consisted of 5 μL of DNA template, 1.5 μL of each primer, 30 μL of GoTaq® Green master mix 2× (Promega, USA) and 12 μL of nuclease-free water. The PCR conditions included an initial denaturation at 95°C for 5 min, followed by denaturation at 95°C for 45 s, annealing at 55°C for 60 s, elongation at 72°C for 90 s and final extension at 72°C for 8 min, totaling 35 cycles. The amplified PCR products were visualized through electrophoresis on a 1.2% agarose gel for 60 min. Subsequently, the PCR products were subjected to sequencing, converted into FASTA format, and compared to the NCBI database by employing the BLASTn algorithm. The phylogenetic tree was constructed using the neighbour-joining method with the assistance of MEGA11 software (Newman *et al.* 2016).

***In vivo* experiment of *F. oxysporum* on red cherry tomatoes**

The artificial inoculation of red cherry tomato fruit was conducted following the modified methods described by Wang *et al.* (2010) and Galitskaya *et al.* (2022). Suspensions of yeast isolates were prepared and adjusted to a final concentration of 10^8 CFU/mL. Spore suspensions of *F. oxysporum* were also prepared and adjusted to a final concentration of 10^6 spores/mL. Red cherry tomatoes were sterilized using 75% ethanol for 30 sec, followed by rinsing with sterile distilled water for 60 sec, and subsequent air-drying. Holes were created uniformly using a sterile cork borer with a diameter of 3 mm and a depth of 2 mm. 20 μL of yeast suspension was inoculated into each hole and after 2 h, 10 μL of spore suspension were pipetted into each hole. The experiment included three replications, with six red cherry tomatoes used for each replication. After 7 days of incubation, the disease incidence was calculated. The growth of the fungus was visually assessed on a scale from 0 to 5, where 0 indicated no signs of decay, 1 indicated the presence of small pathogen colonies, 2 indicated pathogen colonies occupying 30–50% of the hole, 3 indicated pathogen colonies occupying 50–100% of the hole, 4 indicated decay slightly exceeding the hole diameter (10–50% diameter), and 5 indicated decay significantly surpassing the hole diameter (> 50% diameter). The disease

incidence (DI) was calculated using a specific formula (Galitskaya *et al.* 2022):

$$\text{DI}\ (\%) = \frac{\sum_1^5 (DI\ level)\ \text{x Number of tomato at the DI level}}{\text{Total number of tomato in the replication x The highest score (5)}}\ x\ 100$$

Statistical analysis

Data were presented as the means ± standard deviations (SD). All *In vivo* data were analyzed using the statistical software SPSS v25. The normality and distribution were tested using Shapiro-Wilk and Levene's test then continued with the One-Way Analysis of Variance (ANOVA) for normally distributed data, followed by Tukey's test. Differences were considered significant when $P \leq 0.05$. On the other hand, Kruskal-Wallis was used if the data was abnormally distributed.

Results

Growth promoting characteristics of yeast isolates

Out of the 63 yeast isolates tested, 38 demonstrated phosphate solubilization activity, as indicated by SI greater than 1. Among these, 11 yeast isolates exhibited high phosphate solubilization activity with an SI greater than 2 (Table 2). Notably, yeast isolates C9 displayed the highest solubilization index (SI = 2.80) among all 63 isolates. None of the yeast isolates demonstrated a positive response in the ammonia or HCN production tests. However, yeast isolates E8 and E10 exhibited positive results in the IAA production test. Concerning antifungal activities, all 63 yeast isolates displayed inhibition activity against both *F. oxysporum* and *C. beticola*, while 62 isolates displayed inhibition activity against *Al. porri*. The extent of growth inhibition varied, ranging from 4.69 to 53.26% for *F. oxysporum*, 10.98 to 62.96% for *C. beticola* and 0 to 56.67% for *Al. porri* (Table 3). Noteworthy yeast isolates T2, C10 and C3 exhibited the highest antifungal activity against *F. oxysporum*, *C. beticola*, and *Al. porri*, respectively (Table 3).

Molecular identification of potential yeast isolates

Based on the results regarding antifungal activities toward three fungal pathogens, four best isolates were further analyzed by molecular identification using ITS2 gene. According to sequencing results, the yeast species were identified (Table 4). All isolates were observed by phylogenetic tree (Fig. 1).

In vivo experiment of *F. oxysporum* on red cherry tomatoes

Infected fruit treated with yeast suspensions showed lower disease incidence than to the control (Fig. 2). C7 showed the best results compared to three other isolates, with 51.10% disease incidence (Table 5). *F. oxysporum* growth inhibition was indicated by no mycelium excessive growth.

Discussion

Phosphorus, an essential plant nutrient, naturally exists in the soil in insoluble or inorganic forms. Furthermore, phosphorus availability can be influenced by several factors, including organic matter, clay content, soil mineralization, and pH (Penn and Camberato 2019). Microorganisms have several different mechanisms to solubilize phosphate, such as siderophores excretion, enzyme production, and organic acid secretion (Rawat *et al*. 2021). Therefore, the addition of biological agents capable of converting it into a soluble form is crucial to facilitate phosphorus uptake by plants. Adequate phosphorus levels support plant development and metabolism, while its deficiency inhibits plant growth (Sharma *et al*. 2022; Majeed *et al*. 2023). The conducted test revealed that over 60% of the yeast isolates displayed phosphate solubilization activity, denoted by an SI greater than 1 (Table 2). However, only 17.46% of the total isolates exhibited high phosphate solubilization activity (Kumla *et al*. 2020). Research has identified certain yeast species isolated from soil, such as *Rhodotorula aurantiaca* and *Phichia norvegensis*, as having pronounced phosphate solubilization activity with an SI greater than 2. Furthermore, genera such as *Saccharomyces*, *Rhodotorula*, *Klockera* and *Debaromyces* sp. have been categorized as phosphate solubilizing yeasts (Gizaw *et al*. 2017).

None of the 63 yeast isolates tested exhibited positive results in both the ammonia production and HCN tests. Ammonia production is closely associated with nitrogen availability, as ammonia dissolves in water, forming ammonium (NH_4^+), a form easily absorbed by plants. Nitrogen plays a critical role in plant development, metabolism, and the synthesis of amino acids and nucleic acids. It has been observed that yeast commonly isolated from leaves and roots often display positive results in ammonia production tests. For instance, the dominant genus *Candida* spp. isolated from the rhizosphere of cherry tomato has been found to possess ammonia production capabilities (Chen *et al*. 2022). Therefore, the negative results obtained from yeast isolated from flowers in this study can be attributed to their ammonia production ability.

Hydrogen cyanide is a broad-spectrum antimicrobial compound that is closely linked to antifungal activity (Sharma *et al*. 2022). HCN production may also have implications for phosphate availability. HCN can sequester iron, preventing its binding with phosphate and thereby increasing phosphate availability (Rijavec and Lapanje 2016). It has been reported that yeast isolated from fruit seeds exhibit HCN production ability, which explains the negative results obtained in this study (Bright *et al*. 2022).

Out of the 63 yeast isolates tested, two exhibited positive results in the IAA production test. Indole-3-acetic acid is a phytohormone auxin known to influence plant cell division, thereby enhancing plants' ability to uptake soil nutrients (Ahemad and Kibret 2014). Generally, yeast isolated from roots, leaves and fruits tend to demonstrate

Table 1: Yeast isolates from phyllosphere and ornamental plants

Source of plant	Isolate code	Reference
Jasmine (*Jasminum sambac*)	M1, M2, M3, M4, M5, M6	Indriana
Rose (*Rosa chinensis*)	W1, W2, W3, W4, W5	(2019)
Ruellias (*Ruellia tuberosa*)	R	
Champak (*Magnolia champaca*)	C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11	
Alamanda (*Strophanthus gratus*)	A1, A2, A3, A4, A5	
Soka (*Ixora coccinea*)	O1, O2, O3, O4, O5	
Orange Jasmine (*Murraya paniculata*)	K1, K2, K3	
Kananga (*Cananga odorata*)	E1, E2, E3, E4, E5, E6, E7, E8, E9, E10	
Chrysant (*Chrysanthemum indicum* L.)	N1, N2, N3, N4, N5, N6	
Jarum Tujuh Bilah (*Pereskia bleo*)	U1, U2, U3, U4, U5	
Tuberose (*Polianthes tuberosa*)	S1, S2, S3, S4	
Butterfly Pea (*Clitoria ternatea*)	T1, T2	

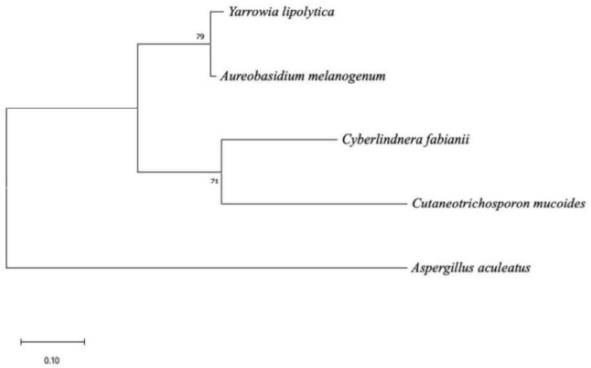

Fig. 1: Phylogenetic tree of the selected yeast isolates

positive results in the IAA production test (Kachalkin *et al*. 2022). Notably, yeast strain *Pichia kudriavzevii* isolated from the mahua flower also displayed a positive result (Patel *et al*. 2017).

Furthermore, the yeast isolates were evaluated for their antifungal activity against the pathogenic fungi *F. oxysporum*, *C. beticola* and *Al. porri*, known to cause fungal diseases in tomatoes (Jain *et al*. 2019). Table 3 presents the yeast isolates with the highest antifungal activity. Several studies have indicated that yeasts can defend against post-harvest diseases and inhibit plant pathogens through the production of secondary metabolites, cell wall-degrading enzymes, and toxins. For instance, *Metschnikowia pulcherrima* has been found to inhibit the fungal pathogen *Botrytis cinerea* by producing pulcherriminic acid, while *Aureobasidium pullulans* can inhibit the fungal pathogens *Alternaria alternata* and *B. cinerea* through the production of polymers, volatiles and secondary metabolites. However, these mechanisms have only been identified in certain species, and the majority of biocontrol yeasts with antagonist activity remain unidentified. Further research and investigations into their adaptations will contribute to a deeper understanding of biocontrol yeasts' abilities to combat plant pathogens (Gouka *et al*. 2022).

C4 was identified as *Cyberlindnera fabianii*, a member of the Basidiomycota group. This yeast species has been utilized in applications such as wastewater treatment and the fermentation of alcoholic beverages (Freel *et al*. 2014). Nonetheless, several studies have highlighted the potential of

Table 2: Phosphate solubilization test of yeast isolates

Code	Average SI ± standard deviation
M2	2.72 ± 0.19
C2	2.35 ± 0.14
C6	2.61 ± 0.19
C9	2.80 ± 0.20
C11	2.08 ± 0.21
A5	2.10 ± 0.09
K1	2.31 ± 0.18
E1	2.01 ± 0.16
U1	2.14 ± 0.29
S2	2.12 ± 0.11
T2	2.07 ± 0.12

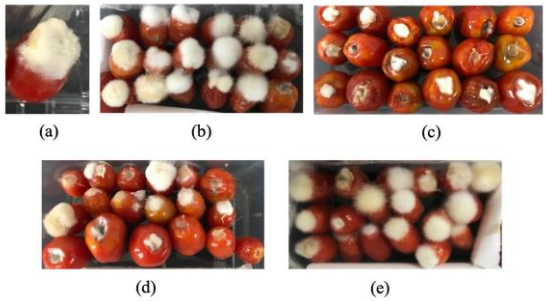

Fig. 2: *In vivo* experiment of *F. oxysporum* on red cherry tomato fruit with selected yeast isolates. (**a**) Sterilized fruit with *F. oxysporum* (control), (**b**) Sterilized fruit with *F. oxysporum* + C4 suspension, (**c**) Sterilized fruit with *F. oxysporum* + C7 suspension, (**d**) Sterilized fruit with *F. oxysporum* + C10 suspension, (e) Sterilized fruit with *F. oxysporum* + K2 suspension

C. fabianii in the field of biofertilizers and agriculture. One study demonstrated that *C. fabianii* strain NRC3 possesses the ability to produce keratinase, a proteolytic enzyme involved in the hydrolysis of insoluble keratin. This finding suggests the potential use of *C. fabianii* as a yeast candidate for developing fertilizers using cost-effective resources such as feather waste from poultry and the leather industry (Abdel-Fattah *et al.* 2015; Li 2022). Likewise, C7 was identified as *Cutaneotrichosporon mucoides*, previously known as *Trichosporon mucoides*, belonging to the Basidiomycota group. Studies have reported the involvement of *C. mucoides* in bioemulsifier production during liquid fermentation systems utilizing sugarcane straw (Cuadrado-Osorio *et al*. 2022). Biosurfactants serve as alternatives to synthetic surfactants and are produced by microorganisms utilizing various substances such as sugar, oil and waste materials (Kee *et al*. 2022). Another study has indicated that *C. mucoides* exhibits relatively high phosphate solubilization ability. However, it is important to note that phosphate solubilization ability does not always correlate with plant growth promotion. Nonetheless, this ability can benefit plants in situations of soluble phosphate deficiency (Vecstaudža *et al*. 2018). This observation aligns with the phosphate solubilization data, which shows that C7 has a solubilization index (SI) of 1.19, higher than that of C4, C10 and K2 with an SI of 1. Thus, *C. mucoides* demonstrates phosphate solubilization activity, while the other three

Table 3: Antifungal activities test of yeast isolates

Pathogens	% Growth inhibition ± standard deviation					
	C3	C4	C7	C10	K2	T2
Fusarium oxysporum	36.73 ± 2.89	48.31 ± 1.20	48.31 ± 1.20	47.17 ± 0.00	48.04 ± 6.93	53.26 ± 1.54
Cercospora beticola	28.33 ± 11.79	58.82 ± 1.66	60.59 ± 0.83	62.96 ± 0.00	59.17 ± 1.18	44.23 ± 2.72
Alternaria porri	56.67 ± 1.57	30.68 ± 1.61	26.14 ± 1.61	44.19 ± 3.29	48.00 ± 11.31	8.00 ± 0.00

Table 4: Molecular identification results

Code	Yeast isolates	Percent identity (%)
C4	*Cyberlindnera fabianii*	99.12
C7	*Cutaneotrichosporon mucoides*	83.84
C10	*Yarrowia lipolytica*	94.77
K2	*Aureobasidium melanogenum*	99.35

Table 5: *F. oxysporum* on cherry tomatoes treated with yeast suspension

Treatment	% Disease incidence
Sterilized fruit with *F. oxysporum* + C4 suspension	90.10^{a}
Sterilized fruit with *F. oxysporum* + C7 suspension	51.10^{ab}
Sterilized fruit with *F. oxysporum* + C10 suspension	64.40^{bc}
Sterilized fruit with *F. oxysporum* + K2 suspension	75.50^{c}

Note: Numbers with different letters illustrate a significant difference at $P \leq 0.05$

isolates do not.

C10 has been identified as *Yarrowia lipolytica*, belonging to the Ascomycota group. *Y. lipolytica* can be found in various habitats, including soil, marine water, consumable products, and even in the excreta of vertebrates or insects that consume them. *Y. lipolytica* also has potential for applications in the field of agriculture, including its use as a biofertilizer. It has been found to effectively act as a post-harvest biocontrol agent, inhibiting blue mold decay in apples caused by the pathogen *Penicillium expansum*. This biocontrol mechanism involves the stimulation of genes responsible for the apple defense system, such as peroxidase, chitinase and polyphenol oxidase. Additionally, *Y. lipolytica* strain FH1 exhibits notable antioxidant activity and can produce significant amounts of IAA, indole-3-acetamide (IAM), flavonoids and phenols. These attributes position *Y. lipolytica* as a promising microorganism for applications in biofertilizer production and the agricultural industry (Mamaev and Zvyagilskaya 2021).

K2 was identified as *Aureobasidium melanogenum*, a member of the Ascomycota group. This yeast species can be found in hypersaline aquatic environments as well as indoor human habitats (Francesco *et al*. 2023). In the field of agriculture, *Au. melanogenum* plays a role in controlling fruit mold diseases to ensure and maintain fruit quality. Studies have shown that *Au. melanogenum* exhibits inhibitory activity against various spoilage molds such as *P. expansum* (blue mold), *B. cinerea* (gray mold) and *Al. alstroemeriae* (necrotic leaf blotch) during both pre- and post-harvest stages of apple production (Gomomo *et al*. 2022). Additionally, *Au. melanogenum* has been found to possess the ability to produce siderophores, which are chelating agents for ferric iron. This ability may contribute to supporting plant growth and indirectly inhibiting plant pathogens by sequestering available iron from the environment. These characteristics highlight the potential of *Au. melanogenum* in various agricultural applications, including the development of biofertilizers (Kumla *et al*. 2020). The phylogenetic tree analysis reveals a closer relationship between *C. mucoides* and *C. fabianii* compared to the other two yeast species (Fig. 1). This can be attributed to their shared classification within the Basidiomycota group. On the other hand, *Au. melanogenum* and *Y. lipolytica* belong to the Ascomycota group.

The *in vivo* experiment utilized the pathogen *F. oxysporum*, which is commonly associated with tomato diseases and can lead to spoilage of *C. beticola* and *Al. porri* were not included in the experiment as they primarily target plants rather than post-harvest fruits. *C. beticola* is responsible for *Cercospora* leaf spot disease in tomatoes (Song *et al*. 2023), while *Al. porri* causes leaf blight disease (Adhikari *et al*. 2021). The results of the *in vivo* experiment can be observed in Table 4, where C7, identified as *C. mucoides*, exhibited the highest inhibition against *F. oxysporum*. The disease incidence associated with *C. mucoides* was 51.10%, which is lower compared to the other three yeast isolates. Notably, there is limited existing research on *C. mucoides* as an antifungal agent against fungal pathogens.

Conclusion

None of the yeast isolates demonstrated ammonia and hydrogen cyanide production ability. However, strains E8 and E10 exhibited IAA production ability. Phosphate solubilization tests revealed that 11 yeasts exhibited high solubilization activity (SI > 2), with C9 displaying the highest solubilization index. Antifungal activity assays demonstrated that 63 yeasts exhibited inhibition ability against *F. oxysporum* and *C. beticola*, with T2 and C10

displaying the highest inhibition percentages, respectively, against these pathogens. Additionally, 62 yeasts showed inhibition ability against *Al. porri*, with C3 exhibiting the highest inhibition percentage. Subsequently, four isolates were identified: C4 as *C. fabianii*, C7 as *C. mucoides*, C10 as *Y. lipolytica*, and K2 as *Au. melanogenum*. *In vivo* experiments conducted on red cherry tomatoes revealed that *C. mucoides* exhibited the greatest inhibition ability against *F. oxysporum*, as indicated by a disease incidence rate of 51.10%. Future research should focus on investigating growth promotion mechanisms and antifungal mechanisms for further understanding.

Acknowledgements

Authors thank Atma Jaya Catholic University of Indonesia who facilitated laboratory facilities in this experiment.

Author Contributions

SM designed the experiment, interpreted the results, reviewed, and edited the manuscript. YG designed the experiments and reviewed the manuscript. VER performed research, analyzed data, and wrote the manuscript.

Conflicts of Interest

All authors declare no conflict of interest for this publication.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

Funding Source

This experiment was supported from personal funds.

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