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Antagonistic activity of *Candida utilis* SCKU1 yeast against crown rot disease of 'Hom Thong' Banana (*Musa acuminata*, AAA group)

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Abstract *Candida utilis* SCKU1 yeast had an antifungal activity on seven isolates of fruit postharvest pathogens ranging from 21.5–49.1% inhibition. It was the most effective antagonist against two pathogens, *Colletotrichum musae* BCm1 causing banana anthracnose and *Lasiodiplodia pseudotheobromae* DL1 causing durian fruit rot. Inhibition zone appearances on co-culture plates were observed on four pathogens including *C. gloeosporioides* DCg1, *C. musae* BCm1, *L. pseudotheobromae* DL1 and *Phomopsis* sp. isolate DP1. The largest zone of inhibition (10.15±0.75 mm wide) was noticed in *L. pseudotheobromae* DL1 plate. Yeast culture filtrate was able to inhibit the mycelial growth of all pathogens, but the most inhibition was found in four pathogens, *C. gloeosporioides* DCg1, *C. gloeosporioides* MCg1, *C. musae* BCm1 and *Phomopsis* sp. isolate DP1 with 80.6–84.2% inhibition. Additionally, both yeast cell suspension and its culture filtrate significantly reduced the disease severity of banana crown rot by 35.9 and 33.5%, respectively. However, the mechanism of antagonistic yeast against crow rot disease was demonstrated by producing cell wall degrading enzymes such as beta-1,3-glucanase and chitinase. Cell suspension and culture filtrate of yeast also provided the induction of disease resistance by stimulating the activity of the defense-related enzyme, phenylalanine ammonia-lyase (PAL), beta-1,3-glucanase and chitinase against the disease. PAL activity was greatly activated by both treatments at 72 h after application (haa). The activity of beta-1,3-glucanase and chitinase was also induced by both treatments starting from 48 haa, but the highest level of both enzyme activities was demonstrated at 96 haa. At 96 haa, the level of chitinase activity was extremely enhanced by culture filtrate of yeast. This preliminary study revealed that either yeast cell suspension or its culture filtrate might be alternatively used for controlling banana crow rot on banana production.

Keywords: Antagonistic yeasts, Cell wall degrading enzymes, Postharvest diseases, Induced disease resistance

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Introduction

Crown rot is an important postharvest disease of all economically important banana cultivars around the world. It is caused by several genera of fungi including *Colletotrichum* spp., *Fusarium* spp., *Verticilliium* spp., *Thielaviopsis paradoxa* and *Lasiodiplodia theobromae* (Ewané *et al*., 2013; Hassan *et al*., 2021; Kamel *et al*., 2016; Lassois *et al*., 2010; Triest and Hendrickx, 2016). In Thailand, the infestation of *L. theobromae* is the most common especially in the 'Hom Thong' banana, which is a variety that is exported and has a high return value. There are several ways to control banana crown rot and its causative fungal agents have been presented in many studies. Chemical control by using fungicides including thiabendazole, imazalil and prochloraz can effectively inhibit the *in vitro* growth of crown rot pathogens including *C. musae*, *L. theobromae* and *Fusarium* species (Johanson and Blazquez, 1992). Essential oils from several medicinal plants including cinnamon, lemongrass, bergamot, cassia and basil have been also found to reduce the severity of banana crown rot and its causative agents, *C. musae, Fusarium* spp. and *L. theobromae* (Kamsu *et al*., 2019; Kulkarni *et al*., 2021; Kulkarni *et al*., 2022). Hot water treatment also has been used to minimize the crow rot disease of bananas by immersing the bananas in hot water at a temperature of 50 ℃ for 20 min, and it can reduce the disease severity by 50% (Alvindia, 2012). Moreover, bananas dipped in hot water combined with salt solution greatly reduce the crown rot disease by up to 88% (Alvindia, 2013b). Additionally, UVC irradiation effectively reduces the severity of banana crow rot, which stimulates plants to produce phenolic compounds and defensive enzymes involved in resistance in plant tissues (Mohamed *et al*., 2017).

Biological plant disease control is another effective method used for crown rot disease management in bananas as presented in several reports. Fungal and bacterial antagonists including *Trichoderma harzianum, Clonostachys byssicola* and *Bacillus amyloliquefaciens* were able to inhibit the growth of crown rot pathogens including *L. theobromae, T. paradosa, C. musae* and *F. verticilliodes* and significantly reduce the disease (Alvindia and Natsuaki, 2008; Alvindia and Natsuaki, 2009; Manigo, 2019; Niroshini and Karunaratne, 2009; Thangavelu *et al*., 2007). Furthermore, the combination treatments between biological control agents and some chemicals such as salt solutions could improve the control of crown rot disease (Alvindia, 2013a, 2013c).

Antagonistic yeasts have been introduced to be a promising biological control agent for the management of banana crown rot disease. Both yeast strains, *Pichia anomala* and *C. oleophila* were able to suppress the *in vitro* growth of either individual pathogen, *C. musae, F. moniliforme* and

Cephalosporium sp. or pathogen complex, and they significantly reduced the crown rot disease caused by a complex of the pathogens (Lassois *et al*., 2008). The yeast antagonists combined with other control measures such as the treatment of calcium chloride and modified atmosphere packaging can enhance the potential for controlling crown rot disease of bananas (Bastiaanse *et al*., 2010). Yeast biocontrol agent, *C. tropicalis* also has been recommended for suppressing anthracnose disease of banana which is caused by *C. musae* (Zhimo *et al*., 2017). Yeast strains also have been widely used in the reduction of other postharvest fruit diseases including fruit rot and blue mold of apple (Chan and Tian, 2005; Han *et al*., 2021; Zhao *et al*., 2021) and fruit decay of lemon (Oztekin and Karbancioglu, 2021). The application of yeast combined with other treatments such as chemicals, plant extract, bio-elicitor and antifungal protein provides a better suppression of postharvest diseases on mango, citrus, kiwi and tomato fruits (Cabral *et al*., 2021; Gao *et al*., 2021; Restuccia *et al*., 2020; Shao *et al*., 2019).

The mechanisms of yeast biocontrol agents against fruit pathogens have been noted in several reports. Competition for nutrients and space over the pathogens is mainly found in yeast *C. tropicalis* against anthracnose pathogen, *C. musae* on banana fruits (Zhimo *et al*., 2017), while yeast, *Wickerhamomyces anomalus* is able to compete with blue mold pathogen, *Penicillium expansum* on apple fruits (Zhao *et al*., 2021). Extracellular enzyme secretion is a major role in both yeasts, *P. membranefaciens* and *Cryptococcus albidus* against fruit rot pathogens, *Monilinia fructicola, P. expansum* and *Rhizopus stolonifera* on apple fruits (Chan and Tian, 2005). Induction of disease resistance in plant tissues by yeast application is frequently observed in a variety of fruit diseases against pathogens including blue mold of apple (Zhao *et al*., 2021), fruit rot of lemon (Oztekin and Karbancioglu, 2021) and fruit rot of kiwifruit (Gao *et al*., 2021).

The published reports on yeasts linked to the suppressing banana crown rot disease were limited. The purpose was to test the efficacy of *C. utilis* SCKU1 yeast cell suspension and culture filtrate on (i) the *in vitro* growth of different postharvest fruit pathogens, (ii) the reduction of banana crown rot disease and (iii) the induction of defense mechanisms in plant tissues against banana crown rot disease.

Materials and methods

Preparation of postharvest fruit pathogens

Seven fungal pathogens were obtained from stock cultures which were isolated from diseased fruits by hyphal tip technique. There were two isolates of

Colletotrichum gloeosporioides DCg1 and MCg1 causing durian anthracnose and mango anthracnose, respectively, an isolate of *C. musae* BCm1 causing banana anthracnose, an isolate of *Lasiodiplodia pseudotheobromae* DL1 causing durian fruit rot, two isolates of *L. theobromae* BL1 and ML1 causing banana crown rot and mango stem-end rot, respectively and an isolate of *Phomopsis* sp. DP1 causing durian fruit rot. All pathogens were already tested for their pathogenicity according to Koch's postulates. The pathogens were grown on potato dextrose agar (PDA) plates and incubated at room temperature (25–28 ℃) for 7 days for further use.

Preparation of yeast culture, yeast cell suspension and yeast culture filtrate

Yeast, *C. utilis* SCKU1 was obtained from Department of Microbiology, Faculty of Science, Kasetsart university, Thailand. Yeast was cultured by streak on PDA medium and incubated for 48 h. The yeast streaked on PDA slants was kept as stock culture. The yeast cell suspension was prepared by pouring 20 mL of sterile distilled water over 48-h-old yeast culture on PDA plate, and yeast cells were scraped using a sterile triangular glass rod. The cell suspension concentration was adjusted to 1x 10^8cells/mL using hemacytometer for further use. For the preparation of yeast culture filtrate, yeast was cultured in 200mL of potato dextrose broth (PDB) medium containing in a 500mL Erlenmeyer flask. Yeast culture was incubated on a shaker at 150rpm at room temperature for 72h and then was filtered through a cellulose membrane (0.45 μ, MERK) for collecting cell-free solution. After this, the solution was centrifuged at 10, 000rpm at 4 ℃, the clear portion was collected as the culture filtrate for further experiment.

Antagonistic activity of yeast against fungal postharvest fruit pathogens

The preliminary efficacy of antagonistic yeast in inhibiting pathogenic fungi was tested by dual culture technique on a 9 cm diameter PDA plates. A 7-day-old fungal agar disk (5 mm in diameter) of each pathogen was separately co-cultured with *C. utilis* SCKU1 yeast on PDA plates. The co-cultured plates were incubated at room temperature for 7 days under a 12 h photoperiod. The PDA plate with pathogen alone was served as control. The radial growth of each pathogen was measured. The experiment was proceeded with 3 replicates and each replicate consisted of 3 plates. The experiment was repeated twice. Percentage of pathogen growth inhibition was calculated according to the formula:

Percentage of inhibition = [(R1 - R2) / R1] x 100

where R1 is the radial growth of the pathogen in control plate and R2 is the radial growth of the pathogen in co-cultured plate.

Culture filtrate of yeast against fungal postharvest fruit pathogens

A PDA agar disk (7 mm in diameter) of each pathogen of a 5-day-old culture was placed at the middle of a sterile plate with a 9 cm diameter. The agar surface with mycelium was faced up. Then, 20 mL of sterile culture filtrate which was filtered through a cellulose membrane syringe filter (0.45 µl, MERK) was added to the plate of each pathogen. The cultures were incubated at room temperature for 7 days under a 12 h photoperiod. Consequently, culture filtrate was removed by filtration using a Whatman No.1 filter paper. The mycelial mats were dried in the oven at 70 ℃ for 6 h and immediately weighed. A mycelial agar disk with PDB medium was served as control. The experiment was operated with 3 replicates and each replicate consisted of 3 plates. The experiment was repeated twice. Percentage of pathogen growth inhibition was calculated with the following formula:

$$\text{Percentage of inhibition} = [(M1 - M2) / M1] \times 100$$

where M1 is the mycelial dry weight of the pathogen in control plate and M2 is the mycelial dry weight of the pathogen in culture filtrate plate.

The sensitivity analysis of culture filtrate was conducted using the percentage values of mycelial growth inhibition plotted with the levels of culture filtrate concentration at 0, 25, 50, 75 and 100%. The value of 50% effective concentration (EC_{50}) of culture filtrate for each pathogen was calculated using the logarithmic regression equation, $y = a \ln(x) + b$.

Preparation of bananas

The mature stage of unripe bananas cv. 'Hom Thong' purchased form the wholesale market was used in this experiment. A healthy banana hand was divided into 4 clusters of 3 to 4 fruits. The cluster of bananas was cleaned with tap water, surface disinfected with 10% Clorox for 5 min, rinsed twice with distilled water, and air dried.

Yeast cell suspension and its culture filtrate against banana crown rot disease

Clusters of bananas were separately sprayed with either yeast cell suspension (1x 10^8cells/mL) or yeast culture filtrate until runoff. The bananas were stored in a moist plastic bag with 85% relative humidity at 25 ℃ for 12 h prior to inoculation with the pathogen. After 12 h, the crown of banana was inoculated with a 7-day-old PDA agar disk (7 mm in diameter) of *L.*

theobromae BL1. Inoculated bananas were continuously incubated at the same conditions for 24 h, and the moisture was consequently released by penetrating the plastic bags. The bananas treated with sterile distilled water and PDB medium were served as negative controls, while the bananas dipped with Luna® Sensation fungicide (10 mL / 20 L, Bayer) was served as a positive control. The experiment was conducted with 3 replicates and each replicate consisted of 3 clusters of bananas. The severity of the disease was assessed at 7 days after inoculation with 5 scoring scales as follows: 0= no discoloration or no pathogen mycelial growth on the crown, 1 = discoloration on the crown and pathogen mycelial growth ≤ 50% of the crown area, 2 = discoloration on the crown and pathogen mycelial growth > 50% of the crown area, 3 = discoloration on the crown and pathogen mycelial growth advanced to finger stalks and 4 = finger stalk and fruit rot occurrence. The percentage of diseases severity reduction was calculated based on the following formula:

$$\text{Percentage of reduction} = [(D1 - D2) / D1] \times 100$$

where D1 is the disease scoring scale of bananas in control treatments and D2 is the disease scoring scale of bananas in treatments of yeast cell suspension and culture filtrate. The experiment was repeated twice.

Cell wall degrading enzymes produced by yeast

The activity of beta-1,3-glucanase was analyzed according to Deshavath *et al*. (2020) and Chanchaichaovivat *et al*. (2008) with modifications using laminarin as a substrate. The laminarin was suspended in 0.05 M sodium acetate buffer, pH 5.0. The yeast culture filtrate (0.5 mL) was mixed with 2 mg/mL laminarin solution. The reaction tube was incubated in a water bath at 35 ℃ for 30 min. Then, the reaction was heated for 10 min and cooled down at room temperature. After that, the solution of 3,5-dinitrosalicylic acid (500 µL) and 0.05 M sodium acetate buffer, pH 5.0 (1.5 mL) was added and mixed well. The reaction tube was heated for 5 min and cooled. The distilled water (2 mL) was added and mixed well prior to reading absorbance at 540 nm using a UV-visible spectrophotometer (CECIL CE7200 Series 7000). Enzyme activity was determined by quantifying the glucose released from laminarin compared to the standard curve of glucose. The enzyme activity was expressed as unit per microgram of protein. The experiment was repeated twice with three replicates.

The activity of chitinase was evaluated following the method of Chanchaichaovivat *et al*. (2008) using swollen chitin as a substrate. The yeast culture filtrate (0.5 mL) was mixed with 1% swollen chitin in 0.05 M citrate-phosphate buffer, pH 6.6 (1 mL). The reaction mixture was incubated in a water bath at 50 ℃ for 60 min, then heated for 5 min and left for precipitation at room temperature. The clear portion was collected for N-acetylglucosamine (NAG)

analysis. The transparent part (500 µL) was mixed with 0.8 M potassium tetraborate solution (100 µL). The reaction tube was heated for 3 min and cooled. The dimethylaminobenzaldehyde solution (3 mL) was added and incubated in a water bath at 37 ℃ for 20 min. After cooling, the absorbance was measured at 585 nm within 10 min compared to standard curve of NGA. The enzyme activity was expressed as unit per microgram of protein. The experiment was repeated twice with three replicates.

The PDB medium which was centrifuged at 10,000 rpm at 4 ℃ was served as a control. The total protein of each enzyme extract was investigated following the method of Bradford (1976), using a standard curve of bovine serum albumin.

Mechanisms of induced resistance in banana fruits

The clusters of bananas were individually sprayed with 50 mL of either yeast cell suspension (1x 10^8cells/mL) or yeast culture filtrate and kept in moist plastic bags at 25 ℃. The bananas sprayed with 0.5 mM salicylic acid, a standard inducer, were used as positive control, whereas untreated bananas were used as negative control. Banana fruits were collected at 0, 24, 48, 72 and 96 h after application and fruit peels were cut into small pieces (1 cm^2 in area). The pieces of fruit peels were wrapped in aluminum foil and then immediately kept at -80 ℃ for further analysis of enzyme activity of phenylalanine ammonia-lyase (PAL), beta-1,3-glucanase and chitinase.

PAL activity assay was conducted following Assis *et al*. (2001) with modifications. The fine powder of a 0.5 g sample obtained by maceration using a mortar and pestle with liquid nitrogen was homogenized in 0.05 M phosphate buffer, pH 8.8 (15 mL) containing β-mercaptoethanol (10 µL). The homogenate was transferred to 5 mL microtubes and centrifuged at 12,000 rpm for 15 min at 4 ℃. The supernatant was collected for PAL activity testing. The reaction tubes contained enzyme extract (200 µL) mixed with 0.05 M borate buffer, pH 8.8, containing 20 mM L-phenylalanine (2.8 mL), and they were incubated at 37 ℃ for 60 min. The reaction was stopped with 1 M HCl (1 mL). Conversion of L-phenylalanine to cinnamic acid was calculated by measuring absorbance at 290 nm compared with standard curve of cinnamic acid solution. The enzyme activity was expressed as unit per microgram of protein. The experiment was repeated twice with three replicates per treatment.

The assay of Velasquez and Hammerschmidt (2004) was used to estimate beta-1,3-glucanse activity. The fine powder of a 0.5 g sample obtained by maceration using a mortar and pestle with liquid nitrogen was homogenized in 0.05 M sodium acetate buffer, pH 5.0 (15 mL) containing 100 mM phenylmethylsulfonyl fluoride (150 µL) and polyvinylpyrrolidone (0.45 g). The

homogenate was transferred to 5 mL microtubes and centrifuged at 12,000 rpm for 15 min at 4℃. The supernatant was used for beta-1,3-glucanse activity assay. The reaction tubes contained enzyme extract (200 µL) mixed with 1 mg/mL laminarin in 0.05 M sodium acetate buffer, pH 5.0 (130 µL). Then, the mixture was incubated at 35℃ for 30 min, subsequently heated for 2-3 min. After that, 3,5-dinitrosalicylic acid (290 µL) and 0.05 M sodium acetate buffer, pH 5.0 (2 mL) were added, and they were heated for 5 min. After cooling, distilled water (1 mL) was added. The beta-1,3-glucanse activity was measured at absorbance of 540 nm. The enzyme activity was expressed as unit per microgram of protein. The experiment was repeated twice with three replicates per treatment.

Chitinase activity was determined using 1% swollen chitin as the substrate following Chanchaichaovivat *et al*. (2008) with some modifications. The supernatant used for detecting beta-1,3-glucanse activity was also used for this enzyme activity assay. The reaction tubes contained enzyme extract (200 µL) mixed with 1% swollen chitin in 0.05 M citrate-phosphate buffer, pH 6.6 (1 mL). The protocol for determining chitinase activity was described above. The enzyme activity was expressed as unit per microgram of protein. The experiment was repeated twice with three replicates per treatment.

The buffer solution used in each enzyme activity assay was served as control. The total protein of each enzyme extract was investigated following Bradford (1976).

Statistical analysis

The experiments were performed twice with a completely randomized design in three replicates. The data from all experiments, except for cell wall degrading enzyme analysis, were evaluated by analysis of variance using SPSS software version 25 (IBM Corp.; USA). Differences between treatment means were determined using Duncan's multiple range test ($p < 0.05$). Data from mycelial growth on the plates, severity score of crown rot disease and lytic enzyme activities were expressed as mean ± standard deviation. The EC_{50} values of yeast culture filtrate against pathogens were evaluated using a logarithmic regression equation in MS Excel 2010.

Results

Antagonistic activity of yeast against fungal postharvest fruit pathogens

The efficacy of yeast, *C. utilis* SCKU1 on inhibiting mycelial growth of seven postharvest pathogens of the fruits on PDA dual culture plates was shown in Table 1. The antagonistic yeast displayed the highest inhibitory effect on *L.*

pseudotheobromae DL1 (durian fruit rot pathogen) by 49.1%, which was not significantly different from the inhibition of *C. musae* BCm1 (banana anthracnose pathogen) (43.8%). Additionally, antagonistic yeast also inhibited the mycelial growth of three isolates of the pathogens including *L. theobromae* ML1 (mango stem-end rot pathogen), *L. theobromae* BL1 (banana crow rot pathogen) and *C. gloeosporioides* MCg1 (mango anthracnose pathogen) by 36.7 – 40%. Moreover, some treatments of co-cultured plates could produce the inhibition zones on PDA surface with various inhibitory activities. The widest inhibition zone was shown on the plate of *L. pseudotheobromae* DL1, followed by the plate of *Phomopsis* sp. isolate DP1 (Table 2 and Figure 1).

Table 1. Mycelial growth inhibition of postharvest pathogens of fruits in dual cultures with Candida utilis SCKU1 yeast, incubated at room temperature (25–28 ℃) for 7 days

Pathogens	Radial mycelial growth of the pathogens (cm)		Mycelial growth inhibition (%)
	Control plates	Dual culture plates	
Colletotrichum gloeosporioides DCg1	7.9±0.31	6.2±0.74	21.5[c1/]
Colletotrichum gloeosporioides MCg1	9.0±0.12	5.7±0.95	36.7[b]
Colletotrichum musae BCm1	8.9±0.42	5.0±0.93	43.8[ab]
Lasiodiplodia pseudotheobromae DL1	8.7±0.78	4.5±0.65	49.1[a]
Lasiodiplodia theobromae BL1	9.0±0.48	5.6±0.37	38.9[b]
Lasiodiplodia theobromae ML1	9.0±0.67	5.4±0.52	40.0[b]
Phomopsis sp. isolate DP1	5.13±0.73	3.83±0.84	25.3[c]

[1/] Means followed by the same letter are not significantly different according to Duncan Multiple Range Test ($P< 0.05$). Means ± standard deviation of 3 replicates are represented.

Table 2. Inhibition zones produced by Candida utilis SCKU1 yeast in dual culture plates against postharvest pathogens of fruits, incubated at room temperature (25–28 ℃) for 7 days

Dual culture plates with *C. utilis* SCKU1 yeast	**Inhibition zone width (mm)**
***Colletotrichum gloeosporioides* DCg1**	1.83±0.24
***Colletotrichum gloeosporioides* MCg1**	–
***Colletotrichum musae* BCm1**	3.74±0.16
***Lasiodiplodia pseudotheobromae* DL1**	10.15±0.75
***Lasiodiplodia theobromae* BL1**	–
***Lasiodiplodia theobromae* ML1**	–
***Phomopsis* sp. isolate DP1**	9.38±0.68

– no inhibition zones. Means ± standard deviation of 3 replicates are represented.

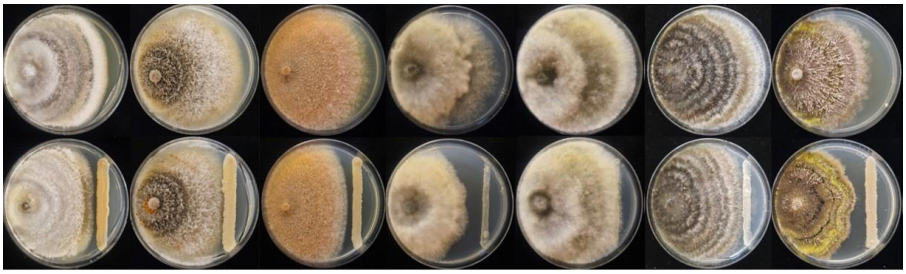

Figure 1. Inhibitory activity of antagonistic yeast, *Candida utilis* SCKU1 against postharvest pathogens of fruits including *Colletotrichum gloeosporioides* DCg1 (A), *C. gloeosporioides* MCg1 (B), *C. musae* BCm1 (C), *Lasiodiplodia pseudotheobromae* DL1 (D), *L. theobromae* BL1 (E), *L. theobromae* ML1 (F) and *Phomopsis* sp. isolate DP1 (G). The plates on the upper panel represent the pathogens grown alone as control treatments; the plates on the lower panel represent the pathogens grown with yeast, incubated at room temperature (25–28 ℃) for 7 days

Culture filtrate of yeast against fungal postharvest fruit pathogens

Culture filtrate of yeast strongly inhibited the mycelial growth of all pathogens. Three isolates of the genera *Colletotrichum* including *C. musae* BCm1 (banana pathogen), *C. gloeosporioides* DCg1 (durian pathogen) and *C. gloeosporioides* MCg1 (mango pathogen) were greatly suppressed by 81.3-84.2%, which were not significantly different from the treatment of *Phomopsis* sp. isolate DP1 (80.6%). Additionally, the rest of three isolates of genera *Lasiodiplodia* including *L. pseudotheobromae* DL1, *L. theobromae* BL1 and *L. theobromae* ML1 was also effectively inhibited by yeast culture filtrate with a 77.2–78.5% reduction (Table 3).

Table 3. Mycelial growth inhibition of postharvest pathogens of fruits using culture filtrate of *Candida utilis* SCKU1 yeast, incubated at room temperature (25–28 ℃) for 10 days

Pathogens	Mycelial dry weight of the pathogens (mg)		Mycelial growth inhibition (%)
	Control plates	Culture filtrate plates	
Colletotrichum gloeosporioides DCg1	118.93±0.93	20.60±0.53	82.7[a1/]
Colletotrichum gloeosporioides MCg1	121.33±0.58	22.63±0.55	81.3 [a]
Colletotrichum musae BCm1	117.80±0.89	18.57±0.60	84.2[a]
Lasiodiplodia pseudotheobromae DL1	120.43±0.59	27.50±0.50	77.2[b]
Lasiodiplodia theobromae BL1	125.53±0.76	27.33±0.47	78.2 [b]
Lasiodiplodia theobromae ML1	121.13±0.81	26.00±0.92	78.5 [b]
Phomopsis sp. isolate DP1	116.20±0.80	22.50±0.78	80.6[ab]

[1/] Means followed by the same letter are not significantly different according to Duncan Multiple Range Test ($P< 0.05$). Means ± standard deviation of 3 replicates are represented.

The sensitivity of the pathogens to yeast culture filtrate was shown in Table 4. The EC_{50} values of culture filtrate for all pathogens were in the range of 52.56– 63.69%. The most effective culture filtrate against the *in vitro* growh of the pathogens was shown in *Phomopsis* sp. isolate DP1, which was the lowest EC_{50} value of 52.56%.

Table 4. The EC_{50} values of culture filtrate of *Candida utilis* SCKU1 yeast against postharvest pathogens of fruits

Pathogens	Logarithmic regression	R^2	EC_{50} (%)
Colletotrichum gloeosporioides DCg1	y = 54.601ln(x) - 176.81	0.8820	63.69
Colletotrichum gloeosporioides MCg1	y = 53.747ln(x) - 171.21	0.9802	61.31
Colletotrichum musae BCm1	y = 53.527ln(x) - 167.38	0.9179	58.03
Lasiodiplodia pseudotheobromae DL1	y = 50.564ln(x) - 158.73	0.9813	62.05
Lasiodiplodia theobromae BL1	y = 52.06ln(x) - 162.41	0.9936	59.15
Lasiodiplodia theobromae ML1	y = 55.152ln(x) - 177.07	0.9910	61.37
Phomopsis sp. isolate DP1	y = 47.539ln(x) - 138.34	0.9996	52.56

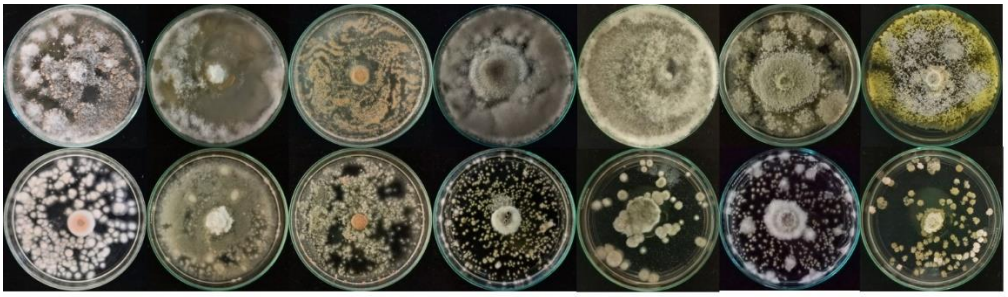

Figure 2. Culture filtrate of antagonistic yeast, *Candida utilis* SCKU1 against postharvest pathogens of fruits including *Colletotrichum gloeosporioides* DCg1 (A), *C. gloeosporioides* MCg1 (B), *C. musae* BCm1 (C), *Lasiodiplodia pseudotheobromae* DL1 (D), *L. theobromae* BL1 (E), *L. theobromae* ML1 (F) and *Phomopsis* sp. isolate DP1 (G). The plates on the upper panel represent the pathogens grown in PDB culture media as control treatments; the plates on the lower panel represent the pathogens grown in yeast culture filtrate, incubated at room temperature (25–28 ℃) for 7 days

Yeast cell suspension and its culture filtrate against banana crown rot disease

The severity of crown rot disease of 'Hom Thong' bananas was significantly reduced by using both yeast cell suspension and its culture filtrate by 35.9 and 33.5%, respectively, compared with the negative control treatments of distilled water and PDB medium, respectively. Unfortunately, both

treatments were less effective in controlling the disease than using the Luna® Sensation fungicide as shown in table 5 and figure 3.

Table 5. Disease severity score of banana crown rot and percentage of disease reduction by using either cell suspension or culture filtrate of antagonistic yeast, *Candida utilis* SCKU1, compared with control treatments at 7 days after inoculation

Treatments	Disease severity score[1/, 2/]	Reduction of disease severity (%)
Yeast cell suspension ($1x10^8$ cells/mL)	2.45±0.02[b]	35.9
Yeast culture filtrate	2.66±0.05[b]	33.5
Luna® Sensation fungicide (Positive control)	1.55±0.02[a]	59.4
Distilled water (Negative control)	3.82±0.12[c]	0.0
Potato dextrose broth (PDB) (Negative control)	4.00±0.09[c]	0.0
CV (%)	32.19	

[1/] Disease severity score: 0 = no discoloration or mycelial growth on the crown, 1 = discoloration on the crown and mycelial growth < 50% of the crown area, 2 = discoloration on the crown and mycelial growth ≥ 50% of the crown area, 3 = discoloration on the crown and mycelial growth advanced to finger stalks, 4 = finger stalk and fruit rot occurrence.
[2/] Means followed by the same letter are not significantly different according to Duncan Multiple Range Test ($P< 0.05$).

Figure 3. The severity of crown rot disease of 'Hom Thong' bananas caused by *L. theobromae* BL1 after treatment with yeast cell suspension (A), yeast culture filtrate (B), Luna® Sensation fungicide (C), distilled water (D) and potato dextrose broth (E), incubated at room temperature (25–28 ℃) for 7 days

Cell wall degrading enzymes produced by yeast

Antagonistic yeast, *C. utilis* SCKU1 was able to produce lytic enzymes, beta-1,3-glucanase and chitinase for drgading the components of pathogen cell wall as shown in Table 6.

Table 6. Enzyme activities of beta-1,3-glucanase and chitinase obtained from 48 h culture filtrate of antagonistic yeast, *Candida utilis* SCKU1 compared with potato dextrose broth

Treatments	Enzyme activity (unit / µg protein)	
	Beta-1,3-glucanase	Chitinase
C. utilis SCKU1 yeast	352.4±0.72	12.2±0.17
Potato dextrose broth (control)	0±0	0±0

Means ± standard deviation of 3 replicates are represented.

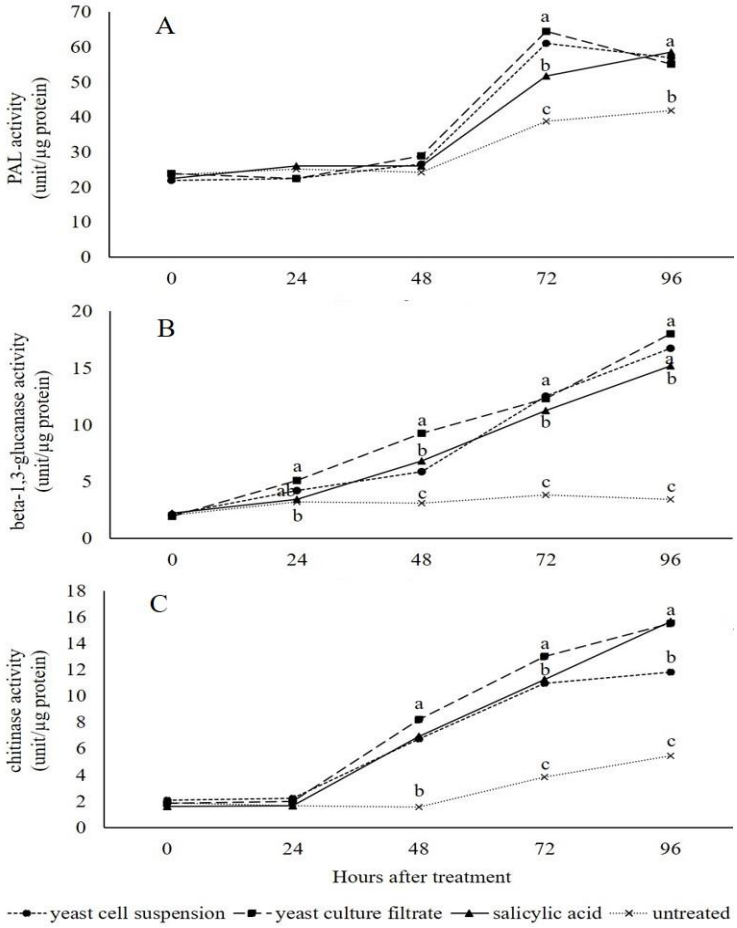

Figure 4. Enzyme activity of phenylalanine ammonia-lyase (A), beta-1,3-glucanase (B) and chitinase (C) on the fruit peel tissues of 'Hom Thong' bananas after treatment with *Candida utilis* SCKU1 yeast cell suspension and its culture filtrate compared with salicylic acid treatment and untreated fruits at different periods, where experiment was repeated twice with three replicates per treatment. Different lowercase letters at each time point indicate significant ($p < 0.05$) different between treatments

Mechanisms of induced resistance in banana fruits

Both treatments of yeast cell suspension and its culture filtrate extremely stimulated the activity of PAL at 72 h after application, which was almost 1.5 fold of untreated fruits. Furthermore, both treatments were able to induce the greater activity of PAL in banana fruit peels than that of 0.5 mM salicylic acid treatment (Figure 4A).

The activity of beta-1,3-glucanase was slightly increased in all treatments until the end of trial period. This enzyme activity was initially stimulated at 24 h by both applications of yeast cell suspension and its culture filtrate, which was significantly different from salicylic acid treated fruits. At 48 h after treatment, culture filtrate of yeast significantly enhanced the beta-1,3-glucanase activity higher than that of yeast cell suspension and salicylic acid applications. The yeast culture filtrate also incessantly activated the level of this enzyme activity until 96 h (Figure 4 B).

All treatments stimulated the level of chitinase activity was observed starting from 24 h after application. A better result in increasing enzyme activity was demonstrated in the fruits treated with culture filtrate of yeast by up to 3 fold of untreated fruits (Figure 4C).

Discussion

The yeast, *C. utilis* SCKU1 was effective in inhibiting the growth of the postharvest pathogenic fungi of fruits including *C. musae* BCm1, *L. theobromae* BL1, *L. pseudotheobromae* DL1, *C. gloeosporioides* MCg1 and *L. theobromae* ML1 by dual culture technique. This study is consistent with a report by Mukherjee *et al*. (2020) which found that various yeast genera such as *Candida* spp., *Cryptococcus* spp., *Metschnikowia* spp. and *Aureobasidium* sp. were effective in controlling the *in vitro* growth of pre- and post-harvest pathogenic fungi. Additionally, Sabaghian *et al*. (2021) reported that three yeasts such as *Starmerella bacillaris*, *Metschnikowia pulcherrima* and *Hanseniaspora uvarum* effectively inhibited the mycelial growth of grapevine pathogens including *Aspergillus* spp., *F. oxysporum*, *Alternaria alternata* and *Phaeomoniella chlamydospora.* Vargas *et al*. (2012) and Rathnayake *et al*. (2018) also reported that several isolates of yeasts isolated from grape leaf surface were able to suppress the growth and spore germination of *Botrytis cinerea* and *Greeneria uvicola*, the causative agents of gray mold and grape rot diseases, respectively.

In this experiment, *C. utilis* SCKU1 yeast was able to produce the inhibition zones against four isolates of the pathogens, *C. gloeosporioides* DCg1, *C. musae* BCm1, *L. pseudotheobromae* DL1 and *Phomopsis* sp. isolate

DP1. This is consistent with a report by Vargas *et al*. (2012) which found that strains of yeast isolated from grapevine surface produced inhibitory regions greater than 4 mm wide on the dual culture plate. Chen *et al*. (2018) also found that some strains of yeasts could produce the zones of inhibitions against *B. cinerea* of strawberry, which were classified as the effective microbial antagonists. Similarly, Jamal *et al*. (2021) demonstrated that thirteen isolates of endophytic yeasts could produce clear zones in dual culture plates against four pathogens including *F. oxysporum, F. solani, Rhizoctonia solani* and *Macrophomina phaseolina,* the causal agents of tomato root rot.

The occurrence of an inhibitory area on culture medium which was produced by yeasts might contain antifungal substances to restrict the growth of the pathogens. Santos *et al*. (2000) reported that *P. membranifaciens* yeast produced killer toxin against the growth of both spoilage yeasts, *Candida boidinii* and *Saccharomyces cerevisiae* with inhibition zone in dual culture plate. Likewise, Souza *et al*. (2021) also demonstrated that several strains of *S. cerevisiae* yeast had an inhibitory activity by producing killer toxins against ocratoxigenic fungi, *Aspergillus ochraceus* and *A. carbonarius* of coffee fruits. Similarly, Maawali *et al*. (2021) investigated that *Meyerozyma guilliermondii* yeast was able to suppress the growth of *A. alternata,* the causal agent of tomato fruit rot with an inhibition zone in culture medium, where the killer toxins such as tricosane and pentacosane were found. However, the application of killer toxin yeasts on edible commodities, particularly postharvest produces should be further investigated for negative effects on human health (Zhang *et al*., 2020).

In this research, *C. utilis* SCKU1 yeast against three pathogens including *C. gloeosporioides* MCg1, *L. theobromae* BL1 and *L. theobromae* ML1 without the inhibition zones was demonstrated. This phenomenon has been frequently found in other previous reports based on the strains of yeasts and the changes of environmental conditions (Feng *et al*., 2021; Hassan *et al*., 2021; Souza *et al*., 2021). Although no inhibition zone in dual culture assay, culture filtrate of *C. utilis* SCKU1 yeast greatly inhibited the mycelial growth of all pathogens in current study. These results were in agreement with Mehalawy (2004) and Francesco *et al*. (2015), reported that culture filtrate of yeasts, *S. unispora, C. steatolytica* and *A. pullulans* had the potential to reduce the *in vitro* growth of *F. oxysporum* causing wilt of bean and *M. laxa* causing fruit rot of peach. Culture filtrate from yeast containing antifungal substances or secondary metabolites against plant pathogens has been reported in several studies. Mehalawy (2004) indicated that culture filtrate of both yeasts, *S. unispora* and *C. steatolytica* consisted of four chemical compounds with retention factor (R_f) 0.1 against *F. oxysporum.* Similarly, Francesco *et al*. (2015) and Hassan *et al*. (2021) found that non-volatile metabolites and diffusible substances from yeasts, *A. pullulans* and *Trichosporon asahii* were effectively restrained the growth of *R. solani* and

C. gloeosporioides, respectively. From our results, *C. utilis* SCKU1 yeast was not the most efficient in controlling the growth of *L. theobromae* BL1 on dual culture assay (38.9% inhibition). It may indicate that the pathogen has a rapid growth rate over the yeast. Contrastingly, yeast culture filtrate had strong ability to inhibit *L. theobromae* BL1 (78.2% inhibition). This assumes that the culture filtrate may contain several extracellular compounds released from the yeast cells which can inhibit the growth of the pathogen *in vitro*.

For controlling the banana crown rot in this testing, both yeast cell suspension and its culture filtrate were able to reduce the disease severity by 33.5–35.9%, which were not comparable to that of fungicide. However, the mechanisms of *C. utilis* SCKU1 yeast against the crown rot disease were evident by the results of the production of cell wall degrading enzymes, beta-1,3-glucanase and chitinase. Both hydrolytic enzymes have been found to be frequently released from antagonistic yeasts for degrading glucan and chitin, respectively, which are parts of the cell wall of the plant pathogenic fungi (Freimoser *et al*., 2019). Chan and Tian (2005) found that both yeasts, *P. membranefaciens* and *Cry. albidus* generated beta-1,3-glucanase and exo-chitinase against fruit rot pathogens of apple, *M. fructicola, P. expansum* and *R. stolonifer*. Capistran *et al*. (2020) also found that some strains of yeasts, *Debaryomyces hansenii* and *Cry. laurentii* produced beta-1,3-glucanase against root rot pathogen of pepper, *Pythium ultimum.* Delali *et al*. (2021) identically demonstrated that three yeasts, *P. kudriavzevii, Kluyveromyces marxianus* and *Yarrowia lipolytica* displayed the ability to produce beta-1,3-glucanase against green mold pathogen of citrus, *P. digitatum.* Furthermore, yeast cells may produce volatile compounds to suppress the pathogens leading to better control of plant diseases (Chen *et al*., 2018; Delali *et al*., 2021; Francesco *et al*., 2015; Maawali *et al*., 2021; Sabaghian *et al*., 2021). Moreover, some strains of yeast antagonists such as *Rhodotorula mucilaginosa, D. hansenii, M. guilliermondii* and *C. orthopsilosis* also have the potential as plant growth promoters, which can produce plant hormones and beneficent substances for plant growth promotion such as indole-3-acetic acid, ammonium and siderophore (Jamal *et al*., 2021; Kareem *et al*., 2021; Mukherjee *et al*., 2020).

Additionally, in this experiment, both cell suspension and culture filtrate of *C. utilis* SCKU1 yeast had the ability to induce resistance to banana crown rot disease by stimulating the activity level of PAL, beta-1,3-glucanase and chitinase. These results were in accordance with other reports showing that antagonistic yeasts, for example, *Cry. albidus* against pod rot of snap beans (Feng *et al*., 2021) *P. membranaefaciens* and *Kloeckera apiculata* against fruit rot of plum (Zhang *et al*., 2019) and *C. oleophila* against fruit rot of kiwi (Gao *et al*., 2021), were able to activate PAL activities in plant tissues. PAL is a key enzyme involved in the phenylpropanoid pathway. It is associated with the

generation of antimicrobial substances such as phenolic compounds and secondary metabolites in plant tissues against plant diseases (Yadav *et al*., 2020). Similarly, the activities of beta-1,3-glucanase and chitinase were stimulated by strains of yeasts, *A. pullulans* and *C. utilis* against anthracnose of banana (Tongsri and Sangchote, 2009). Both beta-1,3-glucanase and chitinase are members of pathogenesis-related proteins, which are capable of being induced by abiotic and biotic factors in the induction of a disease-resistant system (Ali *et al*., 2018). Besides, the yeast antagonists also induced resistance in plant diseases by activating the level of oxidative enzyme activities including catalase, peroxidase, superoxide dismutase and ascorbate peroxidase (Feng *et al*., 2021; Gao *et al*., 2021; Zhang *et al*., 2019; Zhao *et al*., 2021; Zhao *et al*., 2020) and by enhancing phenolic compounds, salicylic acid and jasmonic acid against plant diseases (Jamal *et al*., 2021; Zhao *et al*., 2020). Based on the current study, *C. utilis* SCKU1 yeast had less potential to control banana crown rot. For increasing the potential of yeast, the combination of yeast with other processes has been demonstrated. Several reports showed that yeasts in combination with plant extract, hot water, microbial antagonists and lower dose of chemicals gave more effective in controlling plant diseases than yeast alone (Feng *et al*., 2021; Restuccia *et al*., 2020; Zong *et al*., 2010).

In conclusion, based on dual culture assay, *C. utilis* SCKU1 yeast greatly displayed inhibitory activity on postharvest fruit pathogens, *L. pseudotheobromae* and *C. musae*, which inhibition zones were observed. Culture filtrate of yeast remarkly inhibited the growth of all pathogens. Both yeast cell suspension and culture filtrate of yeast had significant potential to control crown rot disease of bananas. The mechanisms of yeast on reduction of banana crown rot were evident in the production of lytic enzymes, beta-1,3-glucanase and chitinase, and the induction of disease resistance by stimulating the activity levels of defense-related enzyme, PAL and PR-proteins, beta-1,3-glucanase and chitinase against the disease. Further investigation should be evaluated on the potential improvement of *C. utilis* SCKU1 yeast for better control of postharvest diseases.

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