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Antagonistic effect of *Aureobasidium albui* against *Colletotrichum truncatum* causing soybean anthracnose

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Abstract

Soybean anthracnose caused by *Colletotrichum truncatum* is an important disease in Thailand. This study evaluated the antagonistic activity of the epiphytic yeast *Aureobasidium albui* CMU-PPC-172 against *C. truncatum* CMU-PPC-256. The isolates were identified using morphology and phylogenetic analyses of ITS and D1/D2 sequences for *A. albui*, and ITS and GAPDH sequences for *C. truncatum*. In vitro, *A. albui* inhibited pathogen growth by 71.21% and conidial germ tube formation by 100% at 3 h and 45.26% at 6 h. A spot-on-lawn assay also revealed a killer phenotype. On attached soybean leaves under greenhouse conditions, yeast pretreatment prevented lesion development at 3 days after pathogen inoculation. At 5 days, the mean lesion diameter was 0.350 cm in yeast-pretreated leaves compared with 0.718 cm in pathogen-only leaves, representing a 51.25% reduction. Together, these findings demonstrate the antagonistic activity of *A. albui* CMU-PPC-172 against *C. truncatum* CMU-PPC-256 in vitro and its ability to delay lesion development and reduce mean lesion diameter on soybean leaves under greenhouse conditions.

Keywords – Disease management – Eco-friendly crop protection – Green agriculture – Plant-pathogen interactions – Sustainable agriculture

Introduction

Soybean (*Glycine max* L.) is one of the most important legume crops worldwide, serving as a key source of protein and oil for food, feed, and industrial applications (Hartman et al. 2011). In Thailand, soybeans are cultivated primarily in the northern and northeastern regions, with production strongly linked to the expansion of the livestock sector (Panya et al. 2020). However, the country remains a net importer of soybeans, as domestic production is unable to meet demand due to declining cultivation area and productivity constraints (FAO. 2022). Soybean anthracnose, caused mainly by *Colletotrichum truncatum*, is a major disease affecting soybean production across Asia and South America (Cannon et al. 2012). The pathogen can infect all growth stages, causing pre- and post-emergence damping-off, stem lesions, pod rot, and seed decay (Poti et al. 2023). Yield losses can exceed 50% under severe outbreaks (Rogério et al. 2017). In Thailand, *C. truncatum* is widespread in soybean fields, particularly in the northern provinces, where favorable climatic conditions such as high humidity and warm temperatures during the rainy season promote the development and spread of anthracnose disease (Poti et al. 2023).

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Management of anthracnose has heavily relied on chemical fungicides, and in soybeans, management is traditionally dependent on chemical fungicides such as carbendazim, azoxystrobin, difenoconazole, and penthiopyrad (Poti et al. 2023). However, fungicide resistance in *C. truncatum* has been increasingly reported worldwide. Recent studies by Poti et al. (2023) found that *C. truncatum* strains in Thailand are resistant to most of the widely used systemic fungicides. Additionally, excessive fungicide use poses environmental risks, increases production costs, and conflicts with the United Nations Sustainable Development Goals (SDGs) and Thailand's Bio-Circular-Green (BCG) economic model, which emphasize sustainable and eco-friendly agricultural practices.

Biological control using antagonistic microorganisms offers a promising alternative to synthetic fungicides. Several microbial groups, including bacteria (e.g., *Bacillus*, *Pseudomonas*) and fungi (e.g., *Trichoderma*), have shown efficacy in suppressing *Colletotrichum* spp. (Shi et al. 2021). In addition, Yeasts are gaining attention as biocontrol agents due to their ability to colonize plant surfaces, compete for nutrients, and produce antifungal metabolites (Di Francesco et al. 2023). Unlike filamentous fungi, yeasts are generally non-pathogenic, easy to culture, and adaptable to different environmental conditions, making them suitable candidates for formulation into biocontrol products.

Aureobasidium (Ascomycota, Dothideomycetes, Dothideales) is a black yeast-like fungi with considerable potential for biotechnological and agricultural applications. Among *Aureobasidium* spp., *Aureobasidium pullulans* is the most extensively studied for its biocontrol potential and the ability to produce a wide range of bioactive compounds, including melanin (Campana et al. 2022), pullulan, β-glucan, aureobasidin (Singh et al. 2008, Yurlova et al. 1997), siderophores (Li & Chi, 2004), glycerol-liamocin (Kim et al. 2015), exophilins (Price et al. 2013), and volatile organic compounds (VOCs) (Di Francesco et al. 2015, Di Francesco et al. 2020). The broad spectrum of metabolites produced by *Aureobasidium* has increased interest in its potential applications in agriculture, particularly as a source of environmentally sustainable biocontrol agents. The commercial bio-fungicides, such as Botector®, Blossom protect®, consist of two *A. pullulans* strains (DSM 14940 and DSM 14941) are used against fungal and bacterial diseases of grape, strawberry, and pome fruit (Pertot et al. 2017, Sylla et al. 2015).

Direct application of live biological control agents (BCAs) requires intricate formulation steps, rigorous viability checks, field performance monitoring, and ecological impact assessment. Instead, focusing on the microbial secretome allows delivery of defined bioactive components to the infected hosts, enabling more consistent quality control and lower ecological risk (Jaiswal et al. 2022). For example, the antagonistic fungus *Trichoderma harzianum* secretes chitinases and endochitinases when grown on cellulose substrate (Do Vale et al. 2012). The yeast-like biocontrol agent *Aureobasidium pullulans* upregulates secreted hydrolases and esterases during antagonism with *Fusarium oxysporum* (Rueda-Mejia et al. 2021), and the yeast *Pichia kudriavzevii* secretes candidate proteins effective in post-harvest fungal disease control (Elkhairy et al. 2023). This further emphasizes that secretome-based biocontrol is an important future direction for sustainable plant protection.

In the current study, the disease control ability of *Aureobasidium albui* (CMU-PPC-172) against soybean anthracnose-causing *Colletotrichum truncatum* (CMU-PPC-256) was studied. The study revealed that *A. albui* shows higher disease control ability against *C. truncatum*, as demonstrated by in vitro antagonistic assays and disease suppression experiments.

Materials & Methods

Sampling, isolation, and morphology study of the pathogen

Soybean leaf and pod samples with anthracnose symptoms were collected from Doi Saket, Chiang Mai, in July 2025. Symptomatic samples were kept in plastic bags and taken to the laboratory within 24 h. Samples were observed using a Zeiss Stemi 305 stereo microscope, and single spore isolation was performed following Withee et al. (2022). The fungal morphology was observed and photographed using an Axiovision Zeiss Scope-A1 microscope (Zeiss, Jena,

Germany). The fungal isolate *Colletotrichum truncatum* (CMU-PPC-256) used in this study was deposited in the culture collection of the Plant Pathology Division, Faculty of Agriculture, Chiang Mai University (CMU-PPC), Thailand. Measurements were taken with the Tarosoft (R) Image Framework program (Tarosoft, Bangkok, Thailand). The morphological characters were compared with recently published data.

Pathogenicity assay

The pathogenicity assay was performed on healthy, symptomless soybean leaves attached to the one-month-old plants following the methodology in Withee et al. (2022) with modifications. Three plants were used for the inoculation, and one plant was used as a control. Two compound leaves were selected from each plant, with four wounds per leaflet. In the same plant, both wounded and non-wounded tests were performed in two different compound leaves. The experiment was repeated twice. Selected leaves were cleaned using wet tissue paper to remove any impurities or debris. The leaves were then washed with 0.5% NaOCl for 1 min, blotted with sterile tissue paper, and washed three times with sterile distilled water. Each washing step was done for 1 min. Inoculation sites were selected approximately 1 cm from the mid-vein by pricking with a sterile needle to a depth of about 1.0 mm. A conidial suspension of the *C. truncatum* CMU-PPC-256 (1×10^6 conidia mL^{-1}) was prepared in sterile distilled water, and 10 µL of the conidial suspension was used for each inoculation. The control was treated with sterile distilled water. Following inoculation, the plants were maintained at 80% relative humidity under a 12 h light/12 h dark photoperiod until symptom development (Withee et al. 2022).

Selection and morphological characterization of antagonistic yeast

An ongoing survey investigating the antagonistic potential of epiphytic yeasts from different plants in northern Thailand revealed that many epiphytic yeasts are antagonistic against many important plant pathogens (Promthep et al. 2025, Olana et al. 2026). Epiphytic yeasts were initially isolated from fruits and leaves collected from different natural habitats following the method described by Kunyosying et al. (2018). Samples (10 g) were placed in Erlenmeyer flasks containing 50 mL sterile distilled water and shaken at 180 rpm for 15 min to dislodge epiphytic yeasts. Subsequently, 20 µL of each dilution was spread on Yeast Malt Agar (YM agar). The plates were incubated at 28°C for 48 h. Yeast colonies were examined under a microscope, and colonies with different morphological characteristics were selected. The isolates were re-streaked on YM agar to obtain pure cultures and were maintained on YM agar and stored at 4°C until further use.

Several isolates showed promising activity against a variety of fungal phytopathogens, and the isolates with the highest antagonistic potential were deposited in the culture collection of the Plant Pathology Division, Faculty of Agriculture, Chiang Mai University (CMU-PPC) for further studies. For the present study, the yeast strain CMU-PPC-172 was selected from the collection as it showed the highest antagonism against several plant pathogens.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from seven-day-old cultures of *Colletotrichum truncatum* CMU-PPC-256 grown on PDA and three-day-old cultures of the yeast isolate grown on YPD using the FavoPrep Tissue Genomic DNA Extraction Mini Kit (FLAVOGEN, Taiwan) following the manufacturer's protocol. Polymerase chain reaction (PCR) for *Colletotrichum* species was performed to amplify the Internal Transcribed Spacer (ITS) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by using primers ITS1-1F/ITS4 (White et al. 1990) and GDF/GDR (Carbone & Kohn, 1999), respectively. Polymerase chain reaction for yeast was performed to amplify the D1/D2 domain of the large subunit (LSU) rRNA gene and ITS gene using the NL1/NL4 (Lin et al. 1995) and ITS1/ITS4 primer pairs, respectively (White et al. 1990). The PCR conditions for ITS and GAPDH were performed following Damm et al. (2012), and for LSU were performed following Kurtzman (2003). PCR products were sequenced by 1st Base, Malaysia, the commercial sequencing service provider, using Sanger sequencing.

Phylogeny analysis

The quality of the sequences was checked by visualizing the chromatograms using BioEdit. The contig sequences from complementary readings were obtained using SeqMan 5.00. The sequences were checked using NCBI GenBank BLASTn to find the closest representative strains to the isolates. The sequence data for *Aureobasidium* were downloaded from GenBank following the recently published article Jumbam et al. (2025), and the sequence data for *Colletotrichum* were downloaded from GenBank following Wang et al. (2024) (Table 1). Data sets were prepared and edited in BioEdit 7.2 and aligned using MAFFT v7 (Katoh et al. 2019). Single-gene trees were prepared initially to find any incongruences. The concatenated dataset of ITS and GAPDH was used for *Colletotrichum* spp., and the concatenated dataset of ITS and D1/D2 domain was used for *Aureobasidium* spp. The best scoring maximum likelihood (ML) trees were obtained using IQ-TREE 3 (Wong et al. 2026).

Table 1 Taxa used in the phylogenetic analyses and their corresponding GenBank numbers. Culture collection numbers with "*" designate the type. The DNA sequences generated in this study are in bold.

Species	**Culture codes**	**GenBank Accessions**		
		ITS	**D1/D2 domain**	***GAPDH***
Aureobasidium albui	NRRL 61482*	OK376936	OK353650	-
Aureobasidium albui	SA57	OK376939	OK353651	-
Aureobasidium albui	**CMU-PPC-172**	**PZ883120**	**PZ883121**	-
Aureobasidium aubasidani	CBS 100524*	FJ150905	FJ150952	-
Aureobasidium castaneae	CFCC 54591*	MW364284	MW364275	-
Aureobasidium essambei	NRRL 64172*	OK376911	OK353636	-
Aureobasidium melanogenum	CBS 105.22*	FJ150886	FJ150926	-
Aureobasidium melanogenum	CBS 621.80	FJ150885	FJ150921	-
Aureobasidium melanogenum	CDH2020-10	MT863788	MT863787	-
Aureobasidium microtermitis	GTS2.7*	MW276135	MW276136	-
Aureobasidium microtermitis	SRA1.2	MW692853	MW031809	-
Aureobasidium microstictum	CBS 342.66	FJ150903	FJ150945	-
Aureobasidium motuoense	XZY411-4*	OP856710	OP857211	-
Aureobasidium namibiae	CBS 147.97*	FJ150875	FJ150937	-
Aureobasidium pini	CFCC 52778*	MK184533	MK184535	-
Aureobasidium proteae	CBS 114273	JN712491	JN712557	-
Aureobasidium proteae	CPC 13701	JN712490	JN712556	-
Aureobasidium proteae	CBS 111970*	JN712493	JN712559	-
Aureobasidium pullulans	CBS 584.75*	FJ150906	FJ150942	-
Aureobasidium pullulans	CBS 701.76	FJ150907	FJ150951	-
Aureobasidium pullulans	CBS 146.30	FJ150902	FJ150916	-
Aureobasidium pullulans	CBS 100280	FJ150910	FJ150941	-
Aureobasidium rubi	INDP43	OK376878	OK353611	-
Aureobasidium rubi	NRRL 64179*	OK376892	OK353620	-
Aureobasidium subglaciale	EXF 2481*	FJ150895	FJ150913	-
Aureobasidium subglaciale	EXF 3640	FJ150896	FJ150939	-
Aureobasidium subglaciale	EXF-2491	FJ150894	FJ150936	-
Aureobasidium thailandense	NRRL 58539*	JX462674	JX462674	-
Aureobasidium thailandense	NRRL 58543	JX462675	JX462675	-
Aureobasidium vanuatuense	NRRL 64180*	OK376908	OK353633	-
Aureobasidium xishuangbannaense	KUMCC21-0702	ON426834	OP363257	-
Aureobasidium xishuangbannaense	KUMCC21-0703*	ON426835	OP363258	-
Delphinella strobiligena	CBS 735.71	MH860318	MH872074	-
Sydowia polyspora	CBS:116.29	MH855019	MH866487	-
Colletotrichum anthrisci	CBS 125334*	GU227845	-	GU228237
C. chlorophyti	IMI 103806	GU227894	-	GU228286

Table 1 Continued

Species	Culture codes	GenBank Accessions		
		ITS	**D1/D2 domain**	***GAPDH***
C. circinans	CBS 221.81*	GU227855	-	GU228247
C. curcumae	IMI 288937*	GU227893	-	GU228285
C. dematium	CBS 125.25*	GU227819	-	GU228211
C. fructi	CBS 346.37*	GU227844	-	GU228236
C. lineola	CBS 125337*	GU227829	-	GU228221
C. liriopes	CBS 119444*	GU227804	-	GU228196
C. lilii	CBS 186.30	GU227811	-	GU228203
C. phaseolorum 2	CBS 158.36	GU227897	-	GU228289
C. rusci	CBS 119206*	GU227818	-	GU228210
C. spaethianum	CBS 167.49*	GU227807	-	GU228199
C. spinaciae	CBS 128.57	GU227847	-	GU228239
C. tofieldiae	CBS 495.85	GU227801	-	GU228193
C. trichellum	CBS 118198	GU227813	-	GU228205
C. truncatum	CBS 151.35*	GU227862	-	GU228254
C. truncatum	CBS 119189	GU227863	-	GU228255
C. truncatum	CBS 710.70	GU227864	-	GU228256
C. truncatum	CBS 345.70	GU227867	-	GU228259
C. truncatum	**CMU-PPC-256**	**PZ152250**	**-**	**PZ156358**
C. verruculosum	IMI 45525*	GU227806	-	GU228198
C. truncatum	MAP_7	OL851667	-	OL901196
C. truncatum	MSP_13	OL851666	-	OL901195
C. lindemuthianum	CBS 151.28	GU227800	-	GU228192
C. subacidae	MH0565	MZ595874	-	MZ664073
C. acidae	MFLU 18-0233	MG996506	-	MH003692
C. fusiforme	TC20	MT318539	-	MT602199
C. siamens	ICMP 18642	JX010278	-	JX010019
Monilochaetes infuscan	CBS 869.96*	JQ005780	-	JX546612

Antagonistic activity of antagonistic yeast

The antagonistic activity of yeast isolate *Aureobasidium albui* (CMU-PPC-172) was tested against *Colletotrichum truncatum* (CMU-PPC-256) by the co-culture method (Fokkema 1978). Initially, the yeast isolate *A. albui* (CMU-PPC-172) was streaked in a 4 cm straight line on PDA and incubated at 25°C for 72 hours until sufficient yeast growth. Later, a mycelial plug (0.5 mm) from a seven-day-old *C. truncatum* isolate (CMU-PPC-256) was placed 5 cm away from the yeast streak. The mycelial plug placed on a PDA plate without the yeast was used as a control. The experiment was conducted in triplicate. All plates were incubated at 25°C until the control plates were fully grown, reaching the PDA margin on the opposite side of the plate. The radial growth of *C. truncatum* (CMU-PPC-256) and the inhibition zone between the yeast and pathogen were observed, and the percentage of pathogen growth inhibition was calculated using the following formula (Korsten et al. 1995).

Percent inhibition of radial growth PIRG (%) = $[(R1 - R2) / R1] \times 100\%$

R1 = radial growth of the pathogen in the control treatment R2 = radial growth of the pathogen in the yeast treatment

In vitro* adhesion of *Aureobasidium albui* to *Colletotrichum truncatum

The adhesion of *Aureobasidium albui* CMU-PPC-172 to *Colletotrichum truncatum* CMU-PPC-256 was assessed following Pan et al. (2022) with modifications. A 5-mm agar plug from a seven-day-old culture of the pathogen was plated on a fresh PDA plate, 2 cm from the edge. A

sterile glass slide with a 2 × 2 cm PDA block was positioned next to the mycelial plug. The whole setup was incubated for 3 days until the mycelium reached the agar piece on the glass slide. Then, 50 µL of 10^8 cells/mL yeast isolate *A. albui* (CMU-PPC-172) suspension from an overnight yeast peptone dextrose broth (YPD) was flooded onto the mycelia on the agar block. After 24 hrs of incubation, the co-cultured agar block was thoroughly washed to remove excess yeast cells and observed using a microscope to detect whether the yeast cells were strongly attached to the pathogen mycelium.

Inhibitory effect of antagonistic yeasts on conidial germination of *C. truncatum*

The spore germination inhibition assay was performed following the slide culture technique described by Kunyosying et al. (2018) with modifications. 50 µL of the *A. albui* CMU-PPC-172 cell suspension in overnight YPD broth (1×10^8 cells/mL) and 50 µL of conidial suspension from mature *C. truncatum* (CMU-PPC-256) in sterile distilled water (1×10^6 cells/mL) were thoroughly mixed in a sterile microcentrifuge tube. Then, 50 µL of the mixture was spread on PDA plates, while control plates were inoculated with a mixture of conidial suspension and sterile YPD without yeast. From this, a slide culture was prepared using a block of PDA (2 cm × 2 cm) containing yeast and *C. truncatum* spores and maintained in sterile conditions. Conidial germ tube formation was observed and measured at 3, 6, 9, and 12 h.

Presence of the killer phenotype in the antagonistic yeast

The killer phenotype of CMU-PPC-172 was evaluated using a spot-on-lawn assay on buffered YPD–methylene blue agar (YPD–MBA), following the methods described by Schmitt & Breinig (2006) and Abu-Mejdad et al. (2020). *Saccharomyces cerevisiae* was used as the killer-sensitive indicator strain. YPD agar was adjusted to pH 4.5 using citrate–phosphate buffer (10% v/v; 0.1 M citric acid:0.2 M $Na_2HPO_4{\cdot}2H_2O$, 49:51). Methylene blue solution (0.01%, m/v) was sterilized by membrane filtration. The sterile methylene blue solution and 100 µL of an overnight *S. cerevisiae* suspension (1×10^6 cells mL^{-1}) prepared in YPD broth were added to the molten YPD medium, mixed thoroughly, and poured into Petri dishes to prepare the sensitive yeast lawn. The plates were incubated at 20–22°C for 48 h to allow uniform development of the *S. cerevisiae* lawn. Subsequently, three wells (5 mm diameter) were aseptically prepared in each plate using a sterile cork borer. Each well was inoculated with 10 µL of an overnight culture of *A. albui* CMU-PPC-172 grown in YPD broth. For the negative control, 10 µL of sterile YPD broth was added to the wells of plates containing the *S. cerevisiae* lawn. The plates were then incubated at 20–22°C, and the formation of a distinct inhibition zone or dark-blue halo surrounding the wells was examined as an indication of killer activity.

Disease suppression assay on attached soybean leaves under greenhouse conditions

The assay was conducted on attached, unwounded trifoliolate leaves of one-month-old soybean plants under greenhouse conditions. Forty eligible leaves distributed among 30 plants were randomly assigned in a completely randomized design (CRD) to four treatments (10 leaves each): (i) sterile distilled water (negative control), (ii) *Aureobasidium albui* CMU-PPC-172 alone (yeast-only control), (iii) *Colletotrichum truncatum* CMU-PPC-256 alone (pathogen control), and (iv) *A. albui* pretreatment followed by *C. truncatum* inoculation. All three leaflets of each trifoliolate leaf received the same treatment and remained attached throughout the assay.

Two inoculation sites were selected on each leaflet, giving six sites per trifoliolate leaf. An *A. albui* suspension prepared from a three-day-old culture was adjusted to 1×10^8 cells mL^{-1} in sterile 0.85% NaCl. At each site, 10 µL of the yeast suspension was applied to the yeast-only and yeast-pretreated treatments; sterile distilled water was applied to the negative control, and sterile 0.85% NaCl to the pathogen control. After 24 h, 10 µL of a *C. truncatum* conidial suspension (1×10^6 conidia mL^{-1} in sterile 0.85% NaCl) was applied to the pathogen-control and yeast-pretreated sites. The negative-control sites received 10 µL of sterile distilled water; no further suspension was applied to the yeast-only sites. Plants were maintained at approximately 80% relative humidity

under a 12 h light/12 h dark photoperiod. Lesion diameters were measured at 3 and 5 days after pathogen inoculation.

Data analysis

Lesion diameters from the six inoculation sites were averaged to obtain one value per trifoliolate leaf at each assessment day. Results are presented descriptively as treatment means at 3 and 5 days after pathogen inoculation. The percentage reduction in mean lesion diameter following yeast pretreatment was calculated relative to the pathogen-only treatment as:

$$\frac{\text{Pathogen only mean} - \text{Yeast pretreated mean}}{\text{Pathogen-only mean}} \times 100$$

Results

Identification of the pathogen

Morphological study of *Colletotrichum* sp. (CMU-PPC-256) revealed that the isolate highly resembles *Colletotrichum truncatum,* the widely reported causal agent of soybean anthracnose. The leaves and the pods showed disease symptoms such as dry, brown to greyish, slightly sunken necrotic lesions on the surface. The lesion surfaces contained setose oval acervuli. Setae pale to dark brown, mostly smooth, 3–5–septate. Conidiophores hyaline to pale brown, septate. Conidiogenous cells enteroblastic, hyaline to pale brown, cylindrical, 10–20 × 2–4 µm. Conidia hyaline, smooth-walled to verruculose, granular, aseptate, long, curved with a round and truncate base, tapering towards the acute and more strongly curved apex, 20.13–25.33 × 3.15–4.11 µm. Appressoria solitary, light to medium brown, entire to lobed edge, outline roundish to ellipsoidal or clavate. Colonies on PDA cottony, fluffy with distinct, entire margins. Greyish at the beginning and dark green at maturity. Clear concentric circles and concentric pycnidia at maturity with whitish, buff to pale saffron conidia masses (Fig. 1). These characters indicate that our isolate belongs to the *C. truncatum* complex as described in Damm et al. (2009). Further, the phylogenetic analysis of *Colletotrichum* sp. CMU-PPC-256 forms a highly stable clade with *C. truncatum* (Fig. 2). Based on morphological and phylogenetic analyses, we identify CMU-PPC-256 as *C. truncatum.*

Pathogenicity assay

Both wounded and unwounded leaves started developing necrotic lesions with a yellow halo of chlorosis three days after inoculation, while the controls remained healthy. On day five post-inoculation, well-developed necrotic lesions were observed, which were similar to those observed in the field plants used to isolate the suspected pathogen (Fig. 3). Re-isolation of the causal agent from the tested plant resembles the morphology of *Colletotrichum truncatum* detected from the field, fulfilling Koch's postulates. Hence, proving that *C. truncatum* CMU-PPC-256 can cause anthracnose disease in soybeans.

Identification of yeast

The identification of yeast isolate CMU-PPC-172 was performed through morphological and phylogenetic analyses. CMU-PPC-172 produced white to cream, entire, thick, moist, sticky, and slimy colonies on YPD agar. The primary cells 3.94 – 13.59 × 2.4 – 6.35 µm are ovoid to ellipsoid; pseudomycelia were observed, and some of the yeast cells gathered. The morphology of our isolate resembles the *Aureobasidium albui* morphology described in Jumbam et al. 2025 (Fig. 4). The phylogeny of concatenated D1/D2 and ITS revealed that the isolate CMU-PPC-172 forms a phylogenetically stable clade with *A. albui* NRRL 61482, the type for *A. albui* (Fig. 5). Hence, based on morphology and phylogeny, we identify our yeast isolate CMU-PPC-172 as *A. albui.*

Antagonistic activity of the antagonistic yeast

The antagonistic activity of *Aureobasidium albui* (CMU-PPC-172) against *Colletotrichum truncatum* (CMU-PPC-256) was tested using co-culture techniques. The results showed 71.21 %

inhibition of *C. truncatum* (Fig. 6). The test showed a clear inhibition of *C. truncatum* in the presence of *A. albui*. This indicates the ability of *A. albui* to secrete mycelial growth inhibitors against *C. truncatum.* Mycelial thinning of *C. truncatum* was also observed. Interestingly, *A. albui* showed a comparatively faster growth rate and occupied a greater area on the plate.

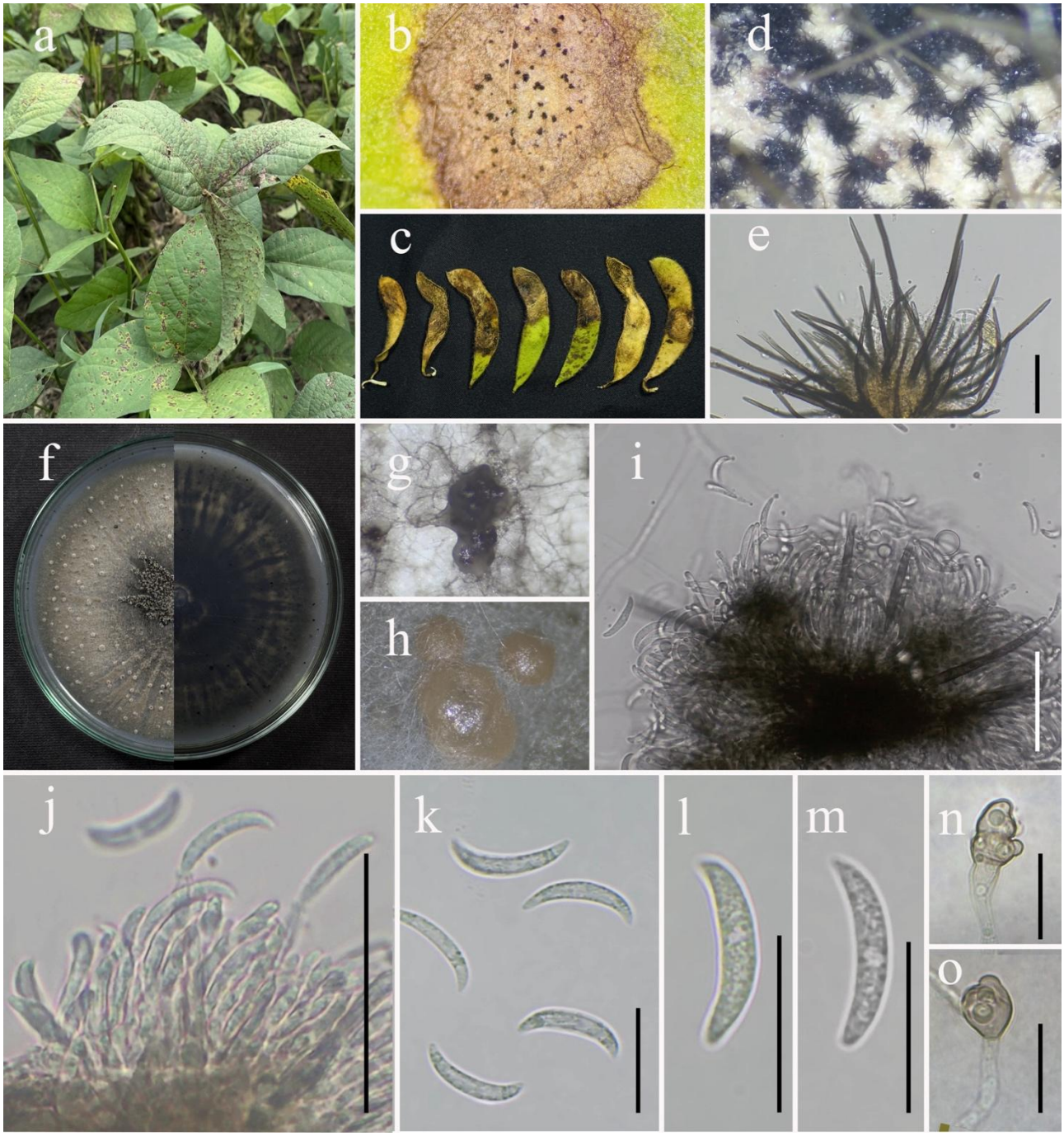

Fig. 1 – Morphology of *Colletotrichum truncatum* (CMU-PPC-256). a, b Symptoms on soybean leaves. c Symptoms on pods. d The acervuli on necrotic lesions. e Acervuli. f Fungal colony on PDA. g, h The conidial mass on PDA. i, j Cross-section of acervuli. k-m Conidia. n, o Appressorium. Scale bars: e = 200 μm; i, k = 50 μm; j, l–o = 20 μm.

In vitro* detection of adhesion by antagonistic yeasts to *Colletotrichum truncatum

Following thorough washing, the treated mycelium was examined to assess the adhesion of *Aureobasidium albui* cells to the pathogen hyphae. Despite repeated washing, yeast cells remained attached to the *Colletotrichum truncatum* mycelium (Fig. 7), indicating a direct physical interaction between *A. albui* and *C. truncatum* hyphae. The persistent attachment of *A. albui* cells to

C. truncatum hyphae suggests a strong physical association between the two microorganisms. Such direct attachment may facilitate antagonistic interactions and could contribute to the observed suppression of *C. truncatum* growth.

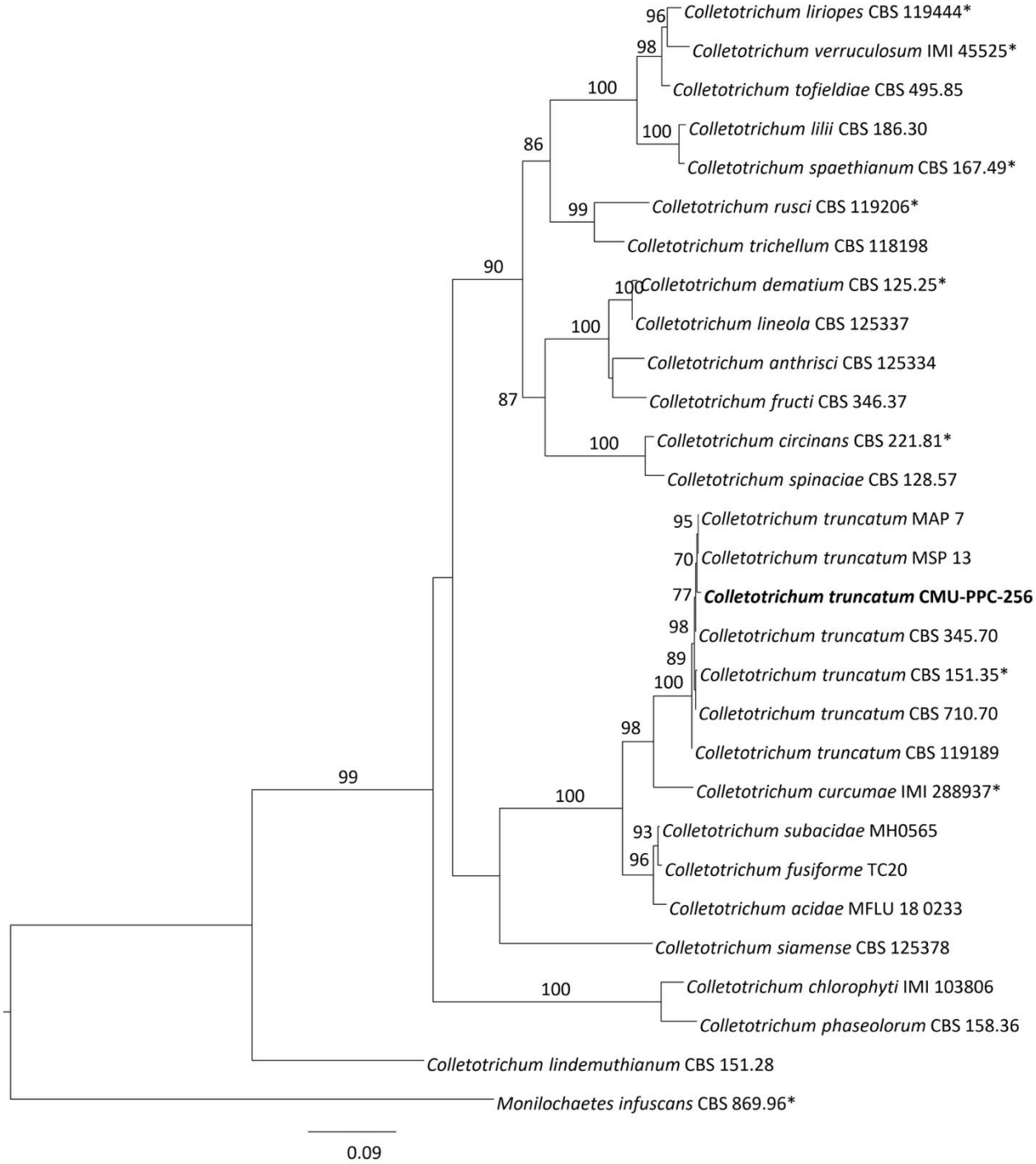

Fig. 2 – Phylogenetic tree constructed by maximum-likelihood analysis of concatenated ITS and GAPDH of *Colletotrichum* spp. Bootstrap support ⩾ 70% was given on the branches. The type strains are marked with *, and the isolate studied in the current study is given in bold.

Germ tube inhibition effects of antagonistic *Aureobasidium albui* against *Colletotrichum truncatum*

The effect of *Aureobasidium albui* on germ tube formation by *Colletotrichum truncatum* was evaluated at 3, 6, and 9 h after treatment. Complete inhibition (100%) of germ tube formation was observed at 3 h after treatment. However, the inhibition gradually decreased to 45.26% and 37.38%

at 6 and 9 h, respectively. These results indicate that the secretome of *A. albui* strongly inhibits germ tube formation by *C. truncatum*, mostly during the early stages of germination (Fig. 8 and 9).

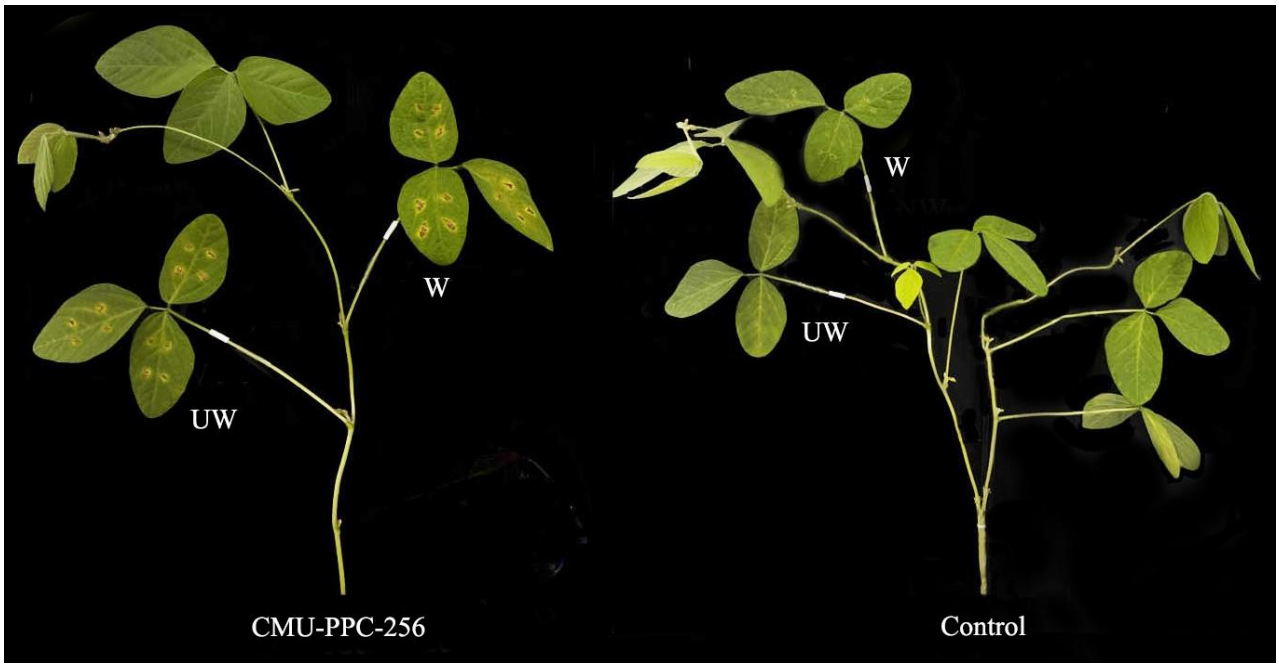

Fig. 3 – Pathogenicity assay of *Colletotrichum truncatum* (CMU-PPC-256) on 30-day-old soybean plants under greenhouse conditions. W = Wounded, UW = Unwounded.

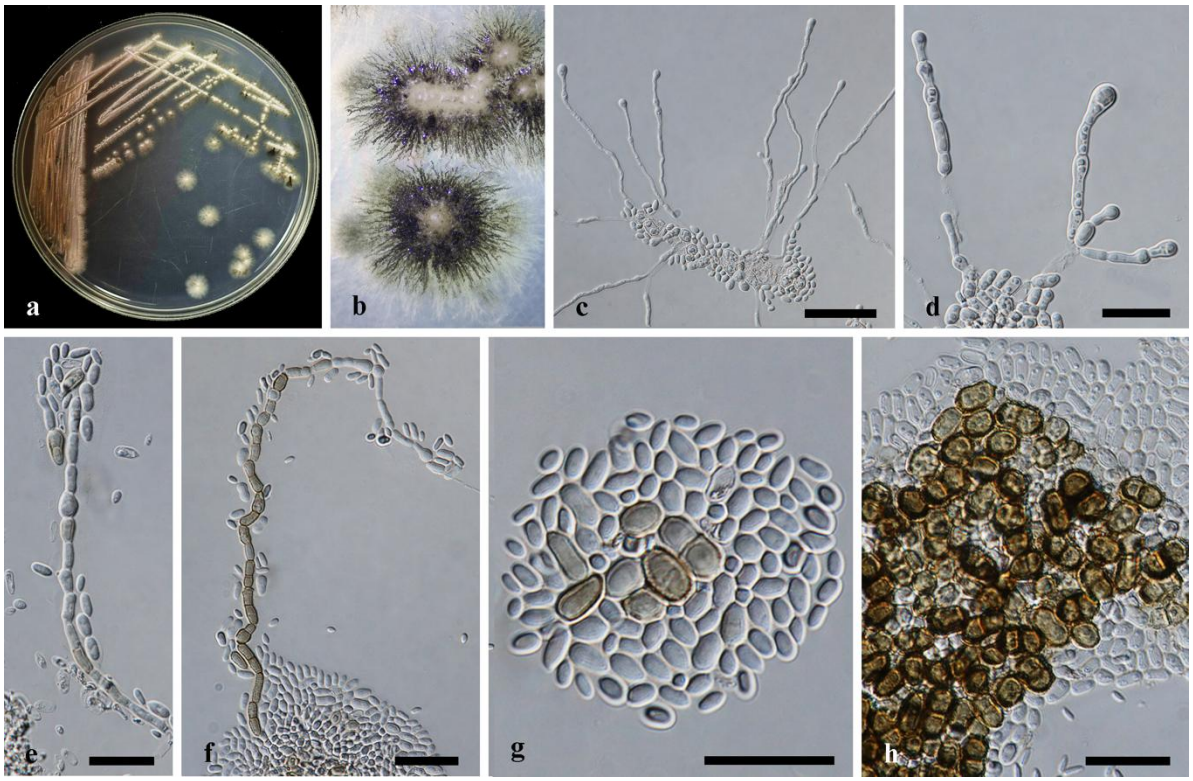

Fig. 4 – Morphological characteristics of *Aureobasidium albui* (CMU-PPC-172). a, b Colony morphology on PDA showing circular, convex colonies with smooth surfaces and a cream to pale whitish coloration. c Pseudohyphae developing from elongating yeast cells. d–h Vegetative yeast cells exhibiting oval to cylindrical morphology. Scale bars = 50 µm (c), 30 µm (d–f), g = 10 µm. h = 20 µm.

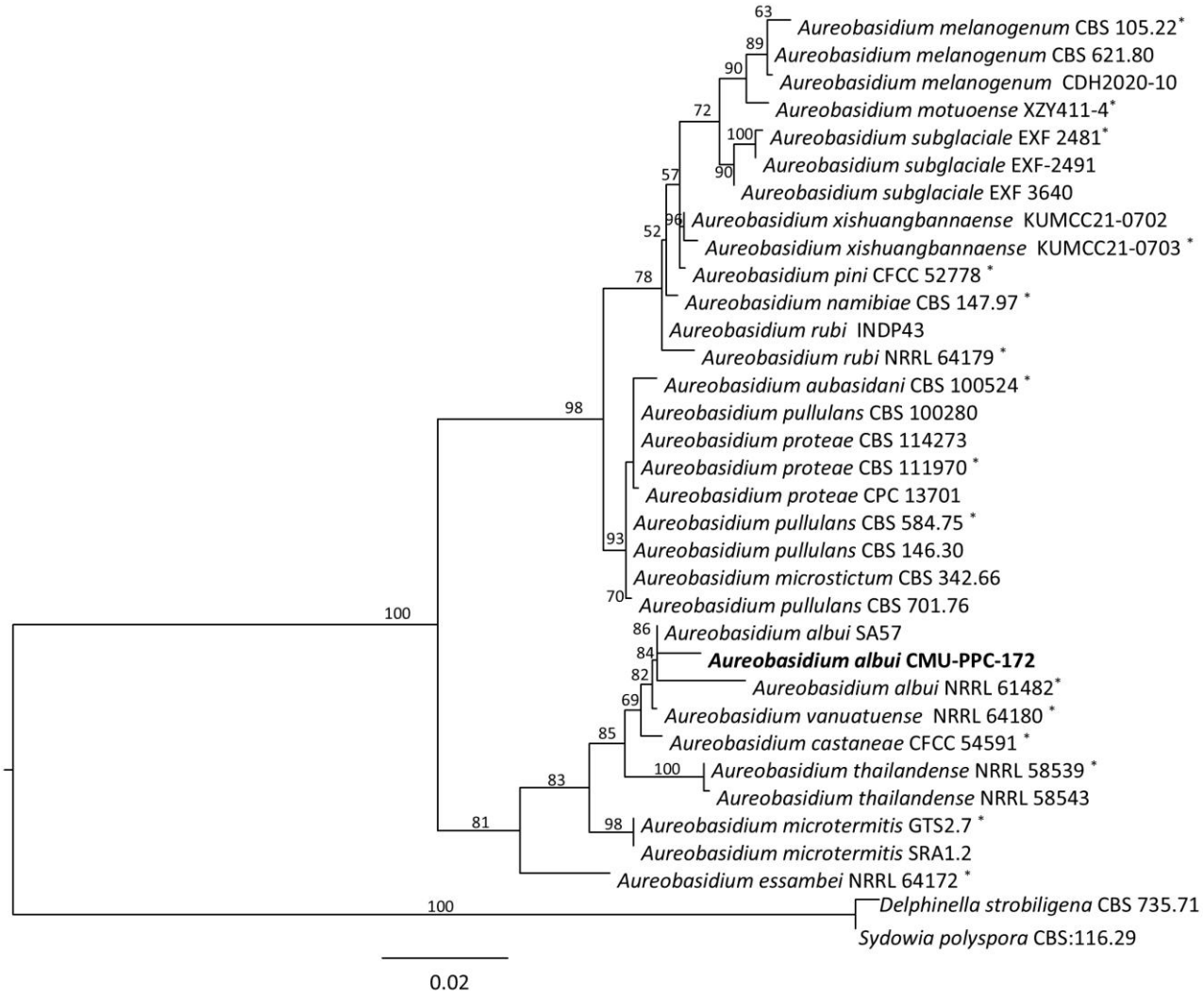

Fig. 5 – Phylogenetic tree constructed by Maximum likelihood analysis of concatenated D1/D2 and ITS of *Aureobasidium* spp. Bootstrap support ⩾ 60% was given on the branches. The type strains are marked with *, and the isolate studied in the current study is given in bold.

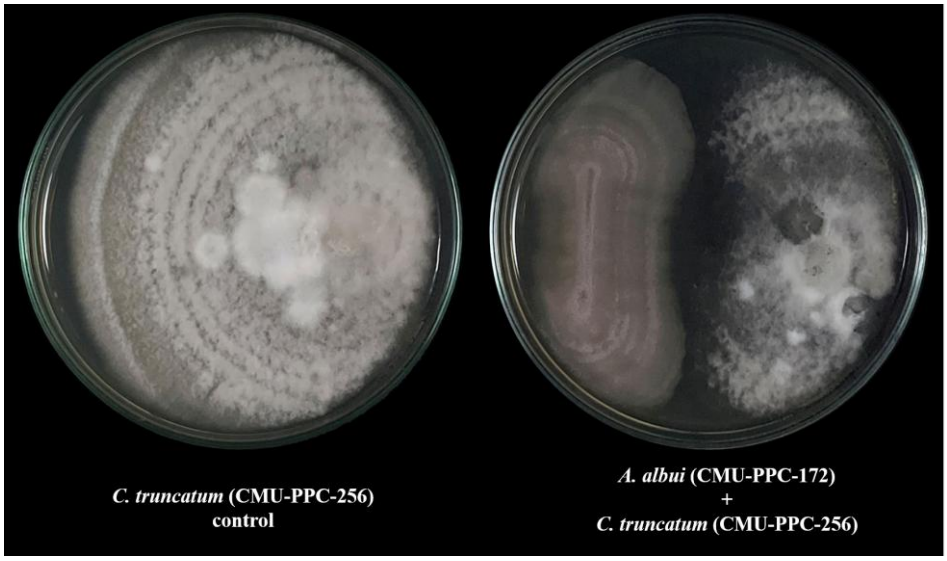

Fig. 6 – Co-culture assay of *Aureobasidium albui* against *Colletotrichum truncatum*.

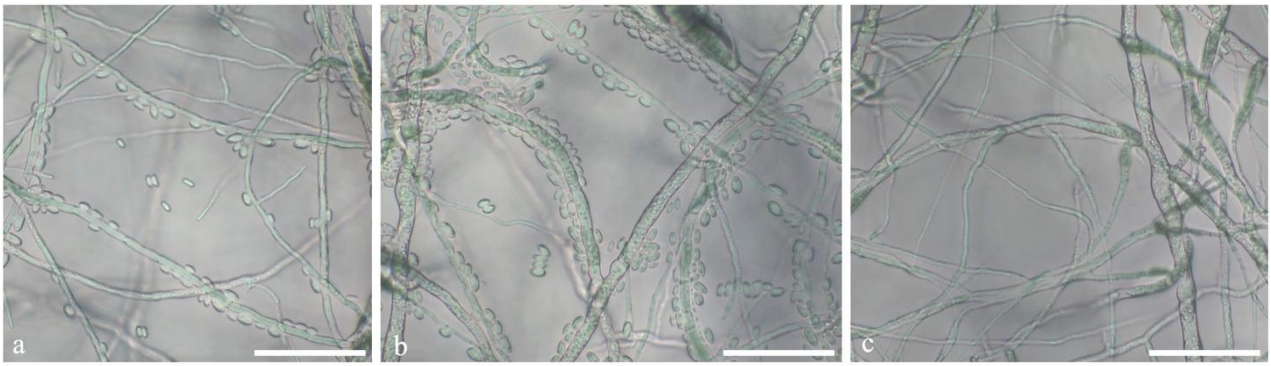

Fig. 7 – Adhesion of *Aureobasidium albui* on *Colletotrichum truncatum.* a, b *C. truncatum* treated with *A. albui.* c Control. Scale bars = 50 µm (a–c).

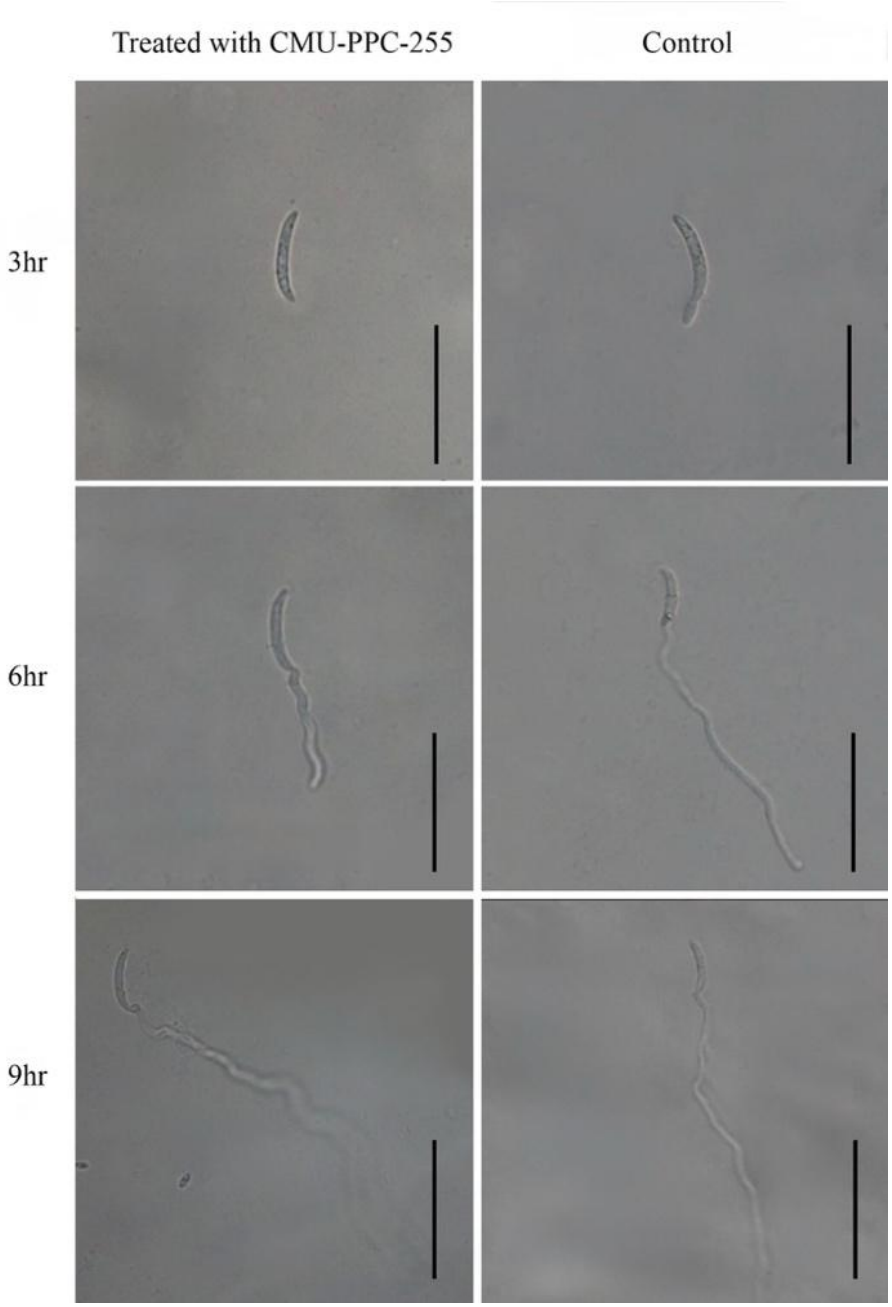

Fig. 8 – Germ tube inhibition effects of antagonistic *Aureobasidium albui* against *Colletotrichum truncatum.* Scale bars = 50 µm.

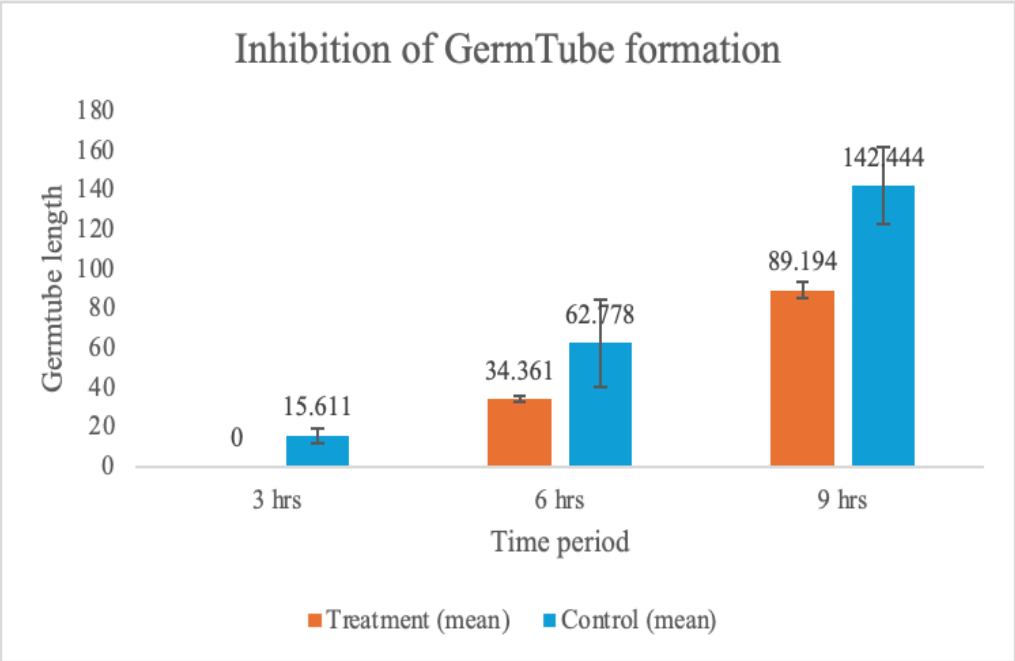

Fig. 9 – The graph of germ tube inhibition effects of antagonistic *Aureobasidium albui* against *Colletotrichum truncatum.*

Presence of a killer phenotype in *Aureobasidium albui*

Certain yeast species exhibit a killer phenotype characterized by the production and secretion of killer toxins that inhibit susceptible microorganisms. In the present study, commercially available *Saccharomyces cerevisiae* was first tested to confirm its sensitivity to killer toxins and was subsequently used as the killer-sensitive indicator strain. Following seven days of incubation, a distinct inhibition halo was observed around the *Aureobasidium albui* colony, indicating the presence of a killer phenotype in *A. albui*. This suggests the production of extracellular antifungal factors, potentially including proteinaceous killer toxins.

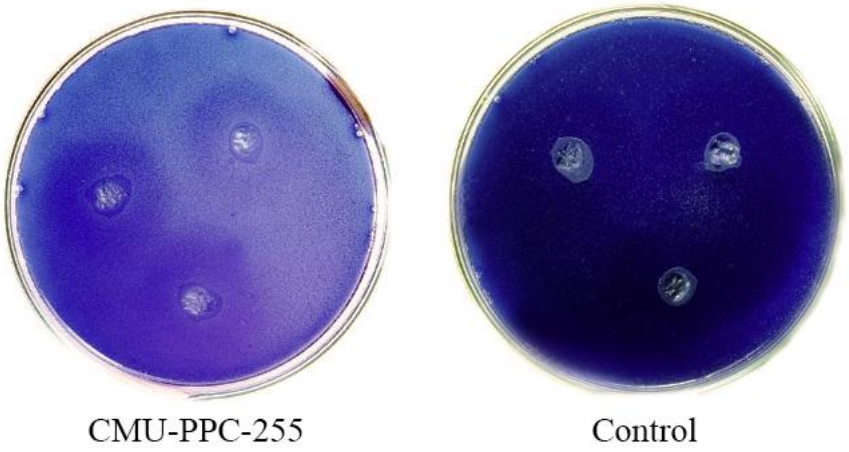

Fig. 10 – Killer toxin assay of *Aureobasidium albui* (CMU-PPC-172) against sensitive *Saccharomyces cerevisiae*.

Disease suppression assay on attached soybean leaves under greenhouse conditions

No lesions developed in the sterile-water or yeast-only controls at either assessment time (Fig. 11a, b). At 3 days after pathogen inoculation, the mean lesion diameter was 0.40 cm in the pathogen-only treatment, whereas no lesions were observed following *Aureobasidium albui* CMU-PPC-172 pretreatment (Fig. 12). At 5 days, the mean lesion diameter was 0.35 cm in the yeast-

pretreated treatment compared with 0.718 cm in the pathogen-only treatment, representing a 51.25% reduction in lesion diameter. Representative symptoms at day 5 are shown in Fig. 11c, d. These results indicate that yeast pretreatment delayed lesion appearance and reduced subsequent lesion development caused by *Colletotrichum truncatum* CMU-PPC-256.

Fig. 11 – Representative attached soybean leaves at 5 days after pathogen inoculation. a Sterile-distilled-water control. b *Aureobasidium albui* CMU-PPC-172 alone. c *Colletotrichum truncatum* CMU-PPC-256 alone. d *A. albui* pretreatment followed by *C. truncatum* inoculation.

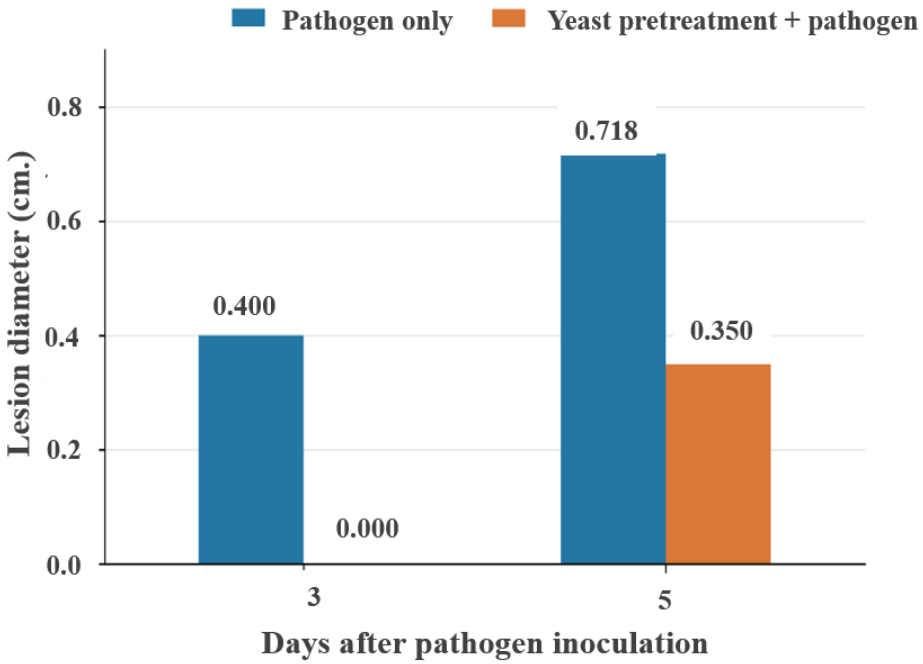

Fig. 12 – Lesion diameter on attached soybean leaves at 3 and 5 days after *Colletotrichum truncatum* inoculation, with or without *Aureobasidium albui* pretreatment. Bars show means of 10 trifoliolate leaves. No lesions developed in the sterile-water or yeast-only controls.

Discussion

Soybean plays a major role in Southeast and East Asian cuisine. Hence, its cultivation and production are important to Thailand's economy (Surya 2024, Taiyawong et al. 2025). However, soybean anthracnose caused by *Colletotrichum truncatum* is becoming a major issue on soybean farms due to the fungicide resistance of *C. truncatum* to the majority of available chemical fungicides (Poti et al. 2023). This shows the importance of seeking novel disease-controlling methods. Biological control of pathogens has become a major topic recently, as it is a sustainable and green option. However, applying living organisms as a controlling agent in the field as biocontrol agents causes many complexities (Jaiswal et al. 2022). Furthermore, it requires a deep understanding of tripartite interactions between the plant, pathogen, and BCA (Karunarathna et al. 2025). Hence, it is worthwhile to examine the potential of the secretome for disease control.

During the current study, the effects of *Aureobasidium albui* against *Colletotrichum truncatum* were studied. Initial co-culture assay revealed that *A. albui* has higher potential for growth suppression of *Colletotrichum truncatum.* With the fast growth of *A. albui, a* clear inhibition zone and mycelial thinning in *C. truncatum* were observed. This indicates the ability of *A. albui* to produce diffusible bioactive compounds. *Aureobasidium* can produce several antifungal metabolites, including liamocins, aureobasidins, lactones, and other secondary metabolites including killer toxins (Freimoser et al. 2019, Moura et al. 2021). Therefore, the observed inhibitory effect of *C. truncatum* by *A. albui* could be due to a plethora of bioactive compounds, viz., enzymes, VOCs, and killer toxins. For efficient biocontrol activity, it is worth exploring the potential of using bioactive compounds directly or in combinations as BCAs.

Previous studies have demonstrated the biocontrol potential of killer yeasts against *Colletotrichum* species. Lopes et al. (2015) reported that several *Saccharomyces cerevisiae* strains (ACB-CR1, ACB-KD1, ACB-CAT1, ACB-BG1, ACB-K1, and ACB-PE2) exhibited antagonistic activity against *Colletotrichum acutatum*, with killer activity and the production of antifungal compounds suggested as possible mechanisms of biocontrol. In the present study, a spot-on-lawn assay using methylene blue as an indicator revealed a distinct inhibition halo around *Aureobasidium albui* CMU-PPC-172 when tested against the killer-sensitive *Saccharomyces cerevisiae* strain. The formation of this halo indicates that *A. albui* CMU-PPC-172 exhibits a killer phenotype and produces extracellular factors capable of inhibiting the growth of the sensitive yeast. Killer activity is not a universal characteristic among yeasts and may vary according to species, strain, and environmental conditions. Therefore, the observed killer phenotype may represent one of the mechanisms contributing to the biocontrol activity of *A. albui* CMU-PPC-172 against *Colletotrichum truncatum*, together with other potential mechanisms such as direct interaction with pathogen hyphae, inhibition of germ tube formation, and production of antifungal metabolites. However, further characterization of the active components is required to confirm whether the observed inhibition is specifically associated with a killer toxin.

Yeasts produce extracellular proteins, such as glycol proteins and killer toxins known as mycocins, suppressing the growth of fungi, bacteria, and protozoans. This could potentially play a major role when used as a biocontrol agent. Specific receptors on the cell wall make mycocin-producing strains immune to their own toxins (Cappelli et al. 2021). This makes killer toxin production more efficient and useful in controlling other fungi or pathogens. Killer toxin production is genetically contingent on dsRNA and synthesizes toxin variants such as K1, K2, and K28 (Melvydas et al. 2016). The K1 and K2 toxins show a similar mode of action. K1 toxin alters cell permeability through binding to the β-1,6-D-glucan present on the cell wall of pathogens. This is facilitated by the cell wall protein Kre1p and creates cation channels. This leads to increased proton permeability and potassium ion leakage in pathogen cells.

The germ tube inhibition assay demonstrated strong inhibition of *Colletotrichum truncatum* germ tube formation by *Aureobasidium albui*, with complete inhibition observed at 3 h and considerable inhibition persisting at 6 h. These findings suggest that extracellular factors produced by *A. albui* may interfere with the germination and early development of *C. truncatum*. In addition to the production of bioactive compounds, direct adhesion may represent another important antagonistic mechanism. *Aureobasidium* species are known to produce extracellular

polysaccharides, including pullulan, which may contribute to their strong adhesive properties (Campana et al. 2022). Consistent with this characteristic, *in vitro* observations in the present study demonstrated strong attachment of *A. albui* cells to *C. truncatum* hyphae, suggesting that direct physical interaction may also contribute to pathogen suppression.

Collectively, the results from the co-culture, germ tube inhibition, adhesion, and killer phenotype assays demonstrate the multifaceted antagonistic potential of *A. albui* CMU-PPC-172 against *C. truncatum*. The observed inhibition suggests that *A. albui* may suppress the pathogen through a combination of direct and indirect mechanisms. In particular, the strong inhibition of germ tube formation and the observed killer phenotype suggest the involvement of extracellular bioactive factors that may interfere with pathogen germination, growth, or establishment. The observed killer phenotype further suggests the presence of extracellular factors with antifungal activity. However, the current assay does not establish whether the inhibitory activity is specifically attributable to a killer toxin or another secreted bioactive compound. Therefore, the chemical identity, stability, and mode of action of the active factors remain to be determined.

The attached-leaf assay showed that yeast pretreatment delayed lesion development, with no lesions observed at 3 days after pathogen inoculation and a 51.25% reduction in mean lesion diameter at day 5. The absence of lesions in the yeast-only control also indicated that yeast application did not cause visible lesions under the tested conditions. These observations complement the in vitro antagonism results and support the potential of *A. albui* for preventive disease management. The appearance of lesions at day 5 indicates that protection was incomplete, highlighting the importance of evaluating application timing and persistence under a wider range of growing conditions.

The combined *in vitro* findings, together with the suppression of disease progression observed in the greenhouse experiment, further support the potential of *A. albui* CMU-PPC-172 as an antagonist of *C. truncatum*. Importantly, these results suggest that secreted bioactive compounds may contribute to the ability of *A. albui* to inhibit pathogen development and establishment. This raises the possibility of exploiting the *A. albui* secretome as an alternative biocontrol approach, potentially reducing the need to apply living microbial cells and thereby facilitating more consistent formulation and quality control. Further investigation of the *A. albui* secretome, including characterization of its bioactive components, stability, spectrum of antifungal activity, and mode of action, is warranted. Such studies could provide a foundation for developing effective and sustainable biocontrol strategies for the management of soybean anthracnose.

References

Abu-Mejdad NMJA, Al-Saadoon AH, Al-Badran AI, Minati MH. 2020 – Optimum conditions of killer toxins produced by *Torulaspora delbrueckii* and *Wickerhamomyces anomalus* and their action as antifungal agents. Bulletin of the National Research Centre 44, 148. Doi 10.1186/s42269-020-00407-8

Campana R, Fanelli F, Sisti M. 2022 – Role of melanin in the black yeast fungi *Aureobasidium pullulans* and *Zalaria obscura* in promoting tolerance to environmental stresses and to antimicrobial compounds. Fungal Biology 126, 817–825.

Cannon PF, Damm U, Johnston PR, Weir BS. 2012 – *Colletotrichum*: current status and future directions. Studies in Mycology 73, 181–213.

Cappelli A, Favia G, Ricci I. 2021 – *Wickerhamomyces anomalus* in mosquitoes: a promising yeast-based tool for the “Symbiotic Control” of mosquito-borne diseases. Frontiers in Microbiology 11, 621605.

Carbone I, Kohn LM. 1999 – A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia, 91(3), 553-556.

Damm U, Cannon PF, Woudenberg JH, Crous PW. 2012 – The *Colletotrichum acutatum* species complex. Studies in Mycology 73, 37–113.

Damm U, Woudenberg JH, Cannon PF, Crous PW. 2009 – *Colletotrichum* species with curved conidia from herbaceous hosts. Fungal Diversity 39(1), 45–87.

Di Francesco A, Ugolini L, Lazzeri L, Mari M. 2015 – Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens. Biological Control 81, 8–14.

Di Francesco A, Zajc J, Gunde-Cimerman N, Aprea E et al. 2020 – Bioactivity of volatile organic compounds by *Aureobasidium* species against gray mold of tomato and table grape. World Journal of Microbiology and Biotechnology 36, 171.

Di Francesco A, Zajc J, Stenberg JA. 2023 – *Aureobasidium* spp.: diversity, versatility, and agricultural utility. Horticulturae 9, 59. Doi 10.3390/horticulturae9010059

Do Vale LH, Gómez-Mendoza DP, Kim MS, Pandey A et al. 2012 – Secretome analysis of the fungus *Trichoderma harzianum* grown on cellulose. Proteomics 12(17), 2716–2728. Doi 10.1002/pmic.201200063

Elkhairy BM, Salama NM, Desouki AM, Abdelrazek AB et al. 2023 – Towards unlocking the biocontrol potential of *Pichia kudriavzevii* for plant fungal diseases*:* in vitro and in vivo assessments with candidate secreted protein prediction. BMC Microbiology 23, 356. Doi 10.1186/s12866-023-03047-w

FAO. 2022 – The State of Food and Agriculture 2022. Leveraging automation in agriculture for transforming agrifood systems. Rome, FAO. Doi 10.4060/cb9479en

Fokkema NJ. 1978 – Fungal antagonisms in the phyllosphere. Annals of Applied Biology 89(1), 115–119.

Freimoser FM, Rueda-Mejia MP, Tilocca B, Migheli Q. 2019 – Biocontrol yeasts: mechanisms and applications. World Journal of Microbiology and Biotechnology, 35(10), p.154.

Hartman GL, West ED, Herman TK. 2011 – Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. Food Security 3, 5–17. Doi 10.1007/s12571-010-0108-x

Jaiswal DK, Gawande SJ, Soumia PS, Krishna R et al. 2022 – Biocontrol strategies: an eco-smart tool for integrated pest and diseases management. BMC Microbiology 22, 324.

Jumbam B, Zasada IA, Bensch K, Crous PW et al. 2025 – A taxonomic revision of *Aureobasidium* (Saccotheciaceae, Dothideales) with new species, new names, and typifications. Persoonia – Molecular Phylogeny and Evolution of Fungi 54, 147–195.

Karunarathna A, Harishchandra DL, Haituk S, Arayapichart S et al. 2025 – Tripartite Interactions in Biocontrol: Insights for Developing Yeast-Based Strategies. Microorganisms 13(10), 2307.

Katoh K, Rozewicki J, Yamada KD. 2019 – MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization, Briefings in Bioinformatics, Volume 20, Issue 4, Pages 1160–1166, Doi 10.1093/bib/bbx108

Kim JS, Lee IK, Yun BS. 2015 – A novel biosurfactant produced by *Aureobasidium pullulans* L3-GPY from a tiger lily wild flower, *Lilium lancifolium* Thunb. PLoS ONE 10, e122917.

Korsten L, De Jager E, De Villiers E, Lourens A et al. 1995 – Evaluation of bacterial epiphytes isolated from avocado leaf and fruit surfaces. Plant Disease, 79(11), 1149-1156.

Kunyosying D, To-Anun C, Cheewangkoon R. 2018 – Control of rice blast disease using antagonistic yeasts. International Journal of Agriculture Technology 14(1), 83–98.

Kurtzman CP. 2003 – Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygotorulaspora*. FEMS Yeast Research 4(3), 233–245.

Li J, Chi Z. 2004 – Siderophores from marine microorganisms and their applications. Journal of Ocean University 3, 40–47.

Lin D, Wu LC, Rinaldi MG, Lehmann PF. 1995 – Three distinct genotypes within *Candida parapsilosis* from clinical sources. Journal of Clinical Microbiology, 33(7), 1815-1821.

Lopes MR, Klein MN, Ferraz LP, da Silva AC et al. 2015 – *Saccharomyces cerevisiae*: A novel and efficient biological control agent for *Colletotrichum acutatum* during pre-harvest. Microbiological Research 175, 93–99.

Melvydas V, Bružauskaitė I, Gedminienė G, Šiekštelė R. 2016 – A novel *Saccharomyces cerevisiae* killer strain secreting the X factor related to killer activity and inhibition of *S. cerevisiae* K1, K2 and K28 killer toxins. Indian Journal of Microbiology 56, 335–343.

Moura VS, Pollettini FL, Ferraz LP, Mazzi MV et al. 2021 – Purification of a killer toxin from *Aureobasidium pullulans* for the biocontrol of phytopathogens. Journal of Basic Microbiology, 61(2), pp.77-87.

Olana RC, Haituk S, Karunarathna A, Cumagun CJR et al. 2026 –. Exploring the biocontrol potential of epiphytic yeasts from fabaceae against *Colletotrichum scovillei* causing chilli anthracnose. Physiological and Molecular Plant Pathology 142, 103114.

Pan H, Zhong C, Wang Z, Deng L et al. 2022 – Biocontrol ability and action mechanism of *Meyerozyma guilliermondii* 37 on soft rot control of postharvest kiwifruit. Microorganisms 10(11), 2143.

Panya K, Joomwong A, Khongbuntad W, Maneewan B. 2020 – Effect of Fermented of Boiled Organic Soybean in Diet on Carcass Composition and Meat Quality of Broiler Chickens. International Scientific Journal of Engineering and Technology 4(1), 6–11.

Pertot I, Giovannini O, Benanchi M, Caffi T et al. 2017 – Combining biocontrol agents with different mechanisms of action in a strategy to control *Botrytis cinerea* on grapevine. Journal of Crop Protection 97, 85–93.

Poti T, Thitla T, Imaiam N, Arunothayanan H et al. 2023 – Isolates of *Colletotrichum truncatum* with resistance to multiple fungicides from soybean in northern Thailand. Plant Disease 107(9), 2736–2750.

Price NP, Manitchotpisit P, Vermillion KE, Bowman MJ et al. 2013 – Structural characterization of novel extracellular liamocins (mannitol oils) produced by *Aureobasidium pullulans* strain NRRL 50380. Carbohydrate Research 370, 24–32.

Promthep T, Haituk S, Nguanhom J, Sittihan S et al. 2025 – Suppression of *Phytophthora cinnamomi* in Avocado Using the Epiphytic Yeast *Pseudozyma hubeiensis*. Journal of Fungal Biology, 15(1), pp.146–158.

Rogério F, Ciampi-Guillardi M, Barbieri MC, Bragança CA et al. 2017 – Phylogeny and variability of *Colletotrichum truncatum* associated with soybean anthracnose in Brazil. Journal of Applied Microbiology 122(2), 402–415.

Rueda-Mejia MP, Nägeli L, Lutz S, Hayes RD et al. 2021 – Genome, transcriptome and secretome analyses of the antagonistic, yeast-like fungus *Aureobasidium pullulans* to identify potential biocontrol genes. Microbial Cell 8(8), 184–202.

Schmitt MJ, Breinig F. 2006 – Yeast viral killer toxins: lethality and self-protection. Nature Reviews Microbiology 4(3), 212–221.

Shi XC, Wang SY, Duan XC, Wang YZ et al. 2021 – Biocontrol strategies for the management of *Colletotrichum* species in postharvest fruits. Crop Protection 141, 105454.

Singh RS, Saini GK, Kennedy JF. 2008 – Pullulan: Microbial sources, production and applications. Carbohydrate Polymers 73, 515–531.

Surya R. 2024 – Fermented foods of Southeast Asia other than soybean-or seafood-based ones. Journal of Ethnic Foods 11(1), 27.

Sylla J, Alsanius BW, Krüger E. 2015 – Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. European Journal of Plant Pathology 143, 461–471.

Taiyawong A, Monkham T, Sanitchon J, Choenkwan S et al. 2025 – Yield stability of soybean variety Morkhor 60 in integrated rotation systems of northeastern Thailand. Plants 14(16), 2503.

Wang T, Wang Y, Geng Z, Wei J et al. 2024 – Identification and pathogenicity of *Colletotrichum truncatum* causing yam anthracnose–A new record in China. Physiological and Molecular Plant Pathology 131, 102246.

White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ

(eds), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA, 315–322.
Withee P, Haituk S, Senwanna C, Karunarathna A et al. 2022 – Identification and pathogenicity of *Paramyrothecium* species associated with leaf spot disease in northern Thailand. Plants 11, 1445.
Wong TK, Ly-Trong N, Ren H, Demotte P et al. 2026 – IQ-TREE 3: phylogenomic inference software using complex evolutionary models. Molecular Biology and Evolution, 43(5), msag 117.
Yurlova NA, de Hoog GS. 1997 – A new variety of *Aureobasidium pullulans* characterized by exopolysaccharide structure, nutritional physiology and molecular features. Antonie van Leeuwenhoek 72, 141–147.