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Effectiveness of *Pseudomonas fluorescens* as an Anthracnose Control Agent in Edamame In Vitro

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Abstract:
Anthracnose caused by *Colletotrichum truncatum* is a major constraint on edamame (*Glycine max* (L.) Merr.) production, and biological control with antagonistic bacteria offers an alternative to synthetic fungicides. This study evaluated the in vitro effectiveness of three concentrations of *Pseudomonas fluorescens* against *C. truncatum*. A non-factorial completely randomized design was used with four treatments (control, 10^7, 10^8, and 10^9 CFU/mL) and five replicates. Antagonism was tested by the dual culture method, and the percentage of growth inhibition was recorded at 24, 48, and 72 h. Colony and hyphal morphology were examined macroscopically and under a light microscope. Means were compared with the Games-Howell test ($p < 0.05$). All concentrations inhibited *C. truncatum* significantly compared with the control but did not differ from each other. The highest mean inhibition was obtained at 10^9 CFU/mL (25.96%), followed by 10^8 CFU/mL (22.66%) and 10^7 CFU/mL (20.80%). Inhibition was highest at 24 h (28.7–35.9%) and declined by 72 h (13.6–22.0%). Pathogen hyphae in the interaction zone showed shrinkage and lysis. *P. fluorescens* has moderate antagonistic activity against *C. truncatum* and deserves further evaluation in planta.

Keywords:
antagonism, biological control, *Colletotrichum truncatum,* dual culture, *Pseudomonas fluorescens*

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INTRODUCTION

Edamame (*Glycine max* (L.) Merr.) is a vegetable soybean harvested at the immature green pod stage. Its pods and seeds are larger and sweeter than those of grain soybean, and the crop is valued as a nutritious food with a higher selling price than most other legumes. In Indonesia, edamame is grown mainly for the fresh and frozen markets, and a large share of the production is exported, particularly to Japan. Export demand is considerably greater than the volume that Indonesia currently supplies, so increasing both yield and pod quality is an important goal for growers and exporters. Reaching this goal depends largely on effective management of diseases that reduce yield and render pods unmarketable.

Anthracnose is one of the most damaging diseases of soybean and edamame. It is caused by several *Colletotrichum* species, among which *C. truncatum* is the most widespread and economically important (Boufleur et al., 2021). The pathogen infects stems, petioles, leaves, and pods and can be transmitted through seed, so infection may occur from the seedling stage until near harvest. Typical

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symptoms are irregular, dark brown to black, sunken lesions that may bear black acervuli. Under warm and humid conditions, conidia are dispersed by rain splash and wind, and the disease can develop rapidly across a field. Yield losses of up to 50% have been reported (Boufleur et al., 2021), and in Brazil and Argentina each 1% increase in anthracnose incidence was associated with a loss of approximately 90 kg of grain per hectare (Dias et al., 2019). For edamame, in which pod appearance determines market value, even small lesions can reduce the proportion of pods that meet export standards.

Control of anthracnose currently relies heavily on synthetic fungicides and seed treatments. Repeated fungicide use increases production costs, can leave residues on fresh pods, harms non-target organisms, and favors the selection of resistant pathogen populations (Mehmood et al., 2023). These problems are particularly relevant for edamame, which is consumed as a fresh vegetable and must meet strict residue limits in export markets. Biological control with antagonistic microorganisms is therefore receiving increasing attention as a safer component of integrated disease management (Nerek and Sokołowska, 2022). Antagonists suppress pathogens through competition for space and nutrients, production of antimicrobial compounds, parasitism, and induction of host resistance, and they generally have a lower environmental impact than chemical pesticides (Gusnadi et al., 2023).

Among bacterial antagonists, fluorescent pseudomonads, particularly *Pseudomonas fluorescens*, are among the most widely studied. These Gram-negative, motile rhizobacteria colonize roots and other plant surfaces efficiently and produce a broad range of bioactive metabolites, including antibiotics, hydrogen cyanide, lytic enzymes, and iron-chelating siderophores (Guzmán-Guzmán and Santoyo, 2022; Rusendra et al., 2024). Many strains also promote plant growth by producing indole-3-acetic acid and solubilizing phosphate (Muthiah et al., 2023), and fluorescent pseudomonads are common members of the rhizosphere community of crop plants (Febriani et al., 2023). *Pseudomonas* is one of the most frequently isolated genera of nodule endophytes, and soybean is the most commonly reported host of such endophytes (Hnini and Aurag, 2024), which suggests that this group is ecologically compatible with leguminous crops. A review of fluorescent pseudomonads concluded that they are effective against a wide range of fungal, bacterial, and viral plant diseases (Okwisan et al., 2023).

Several in vitro studies have shown that *P. fluorescens* can suppress *Colletotrichum*. Ramdan et al. (2021) reported that *P. fluorescens* inhibited the growth of *Colletotrichum* sp. from chili by 36.08% in a dual culture assay, and phylloplane bacteria have been shown to inhibit *Colletotrichum* growth and cause mycelial malformation (Widiantini et al., 2024). However, most of these studies used a single inoculum density and evaluated inhibition at only one time point. The concentration of a bacterial antagonist determines its initial population size and, potentially, the number of antifungal metabolites it produces; it is also an important practical consideration when biological agents are formulated and applied. Information on how the concentration of *P. fluorescens* affects its antagonism against *C. truncatum* from edamame, and how this antagonism changes during the early stages of the interaction, is still limited. In addition, the pathogen's response during the first days of contact with the antagonist is important because this period corresponds to conidial germination and initial colonization of host tissue, when biological agents are expected to act most effectively. Knowing whether inhibition is maintained or lost over time also helps to determine whether an antagonist acts mainly by killing the pathogen or by temporarily slowing its growth, which has direct consequences for the timing and frequency of field applications. Such information is needed before the bacterium can be tested in planta and in the field.

Therefore, this study aimed to evaluate the in vitro effectiveness of *P. fluorescens* at concentrations of 10^7, 10^8, and 10^9 CFU/mL in inhibiting the growth of *C. truncatum*, the causal agent

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of edamame anthracnose. Specifically, the study (i) compared the percentage of growth inhibition among the concentrations at 24, 48, and 72 h of incubation, (ii) described the macroscopic and microscopic characteristics of the pathogen and the antagonist, and (iii) examined morphological changes in the pathogen hyphae in the interaction zone. We hypothesized that inhibition would increase with increasing bacterial concentration.

METHOD

The research was conducted at the Plant Protection Laboratory, Department of Agricultural Production, Politeknik Negeri Jember, East Java, Indonesia, from June to December 2025. The study was a laboratory experiment arranged in a non-factorial completely randomized design (CRD). The sole factor was the concentration of *P. fluorescens*, with four levels: P0 = control without the antagonist; P1 = 10^7 CFU/mL; P2 = 10^8 CFU/mL; and P3 = 10^9 CFU/mL (Table 1). Each treatment was replicated 5 times, with one Petri dish serving as the experimental unit, yielding a total of 20 dual-culture plates. The concentration range was chosen because densities of 10^7–10^9 CFU/mL are commonly used for bacterial biological control agents. Plates were assigned to treatments at random and placed in the incubator at random to minimize positional effects.

Table 1. Treatments of P. fluorescens concentration used in the dual culture assay

Code	Treatment	Bacterial concentration
P0	Control (sterile distilled water, without antagonist)	–
P1	*P. fluorescens*	10^7 CFU/mL
P2	*P. fluorescens*	10^8 CFU/mL
P3	*P. fluorescens*	10^9 CFU/mL

Note: Each treatment was replicated five times (n = 5), giving 20 Petri dishes in total.

The data were analyzed according to the linear model of a non-factorial CRD: $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, where Y_{ij} is the inhibition percentage in the *j*-th replicate of the *i*-th concentration, μ is the general mean, τ_i is the effect of the *i*-th concentration, and ε_{ij} is the random error.

The *C. truncatum* isolate was obtained from edamame plants showing typical anthracnose symptoms and maintained on potato dextrose agar (PDA). The identity of the pathogen was confirmed based on its colony characteristics and conidial morphology. The *P. fluorescens* isolate was obtained from the culture collection of the Plant Protection Laboratory, Politeknik Negeri Jember, and maintained on King's B medium, on which fluorescent pseudomonads produce a yellow-green, fluorescent pigment under UV light. Before the assay, both organisms were subcultured on fresh media: the pathogen was grown on PDA for 7 days, and the bacterium on King's B/nutrient agar for 48 h at 27 ± 2 °C.

Bacterial cells were harvested from the fresh culture and suspended in sterile distilled water. The density of the stock suspension was adjusted to approximately 10^9 CFU/mL using the McFarland standard, and the 10^8 and 10^7 CFU/mL suspensions were prepared by tenfold serial dilution of the stock in sterile distilled water. The viable density of the suspensions was checked by the total plate count method described below.

The antagonism test was carried out by the dual culture method with modifications (Ramdan et al., 2021; Widiantini et al., 2024). Nutrient agar (NA) medium was poured into 9-cm-diameter Petri dishes. The *P. fluorescens* suspension of each concentration was applied by streaking a loopful / by placing a sterile 6-mm paper disc soaked in the suspension 3 cm from the edge of the dish. A 5-mm mycelial plug of *C. truncatum* was then taken from the margin of an actively growing colony using a

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sterile inoculation needle and placed on the opposite side, also 3 cm from the edge of the dish (Figure 1). In the control (P0), the pathogen was placed in the same position, and sterile distilled water was applied in place of the bacterial suspension. All plates were incubated at 27 ± 2 °C in the dark.

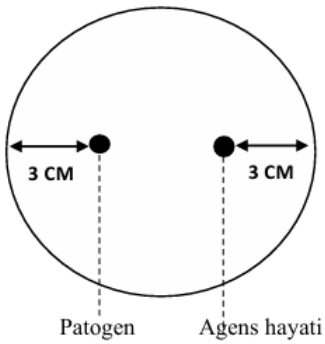

Figure 1. Schematic of the placement of *P. fluorescens* and *C. truncatum* in the dual culture assay

The radius of the pathogen colony was measured at 24, 48, and 72 h after incubation using a digital caliper. Two radii were recorded on each plate: the radius growing away from the antagonist and the radius growing toward it. The percentage of inhibition was calculated using the following formula (Ramdan et al., 2021):

$$\text{Inhibition (\%)} = \frac{r1-r2}{r1} \times 100\%$$

Where r_1 is the radius of the *C. truncatum* colony growing away from *P. fluorescens* (mm) and r_2 is the radius of the colony growing toward *P. fluorescens* (mm). In the control plates, which contained no antagonist, the colony grew radially and symmetrically, so the inhibition was zero. The variables observed in the study are summarized in Table 2.

Table 2. Observed variables, units, and methods of observation

Variable	Unit	Method of observation	Time
Inhibition of pathogen growth	%	Radii r1 and r2 of the pathogen colony; formula (r1 − r2)/r1 × 100%	24, 48, and 72 h
Colony characteristics	–	Macroscopic observation of color, texture, shape, surface, and growth	End of incubation
Hyphal and conidial morphology	–	Light microscopy with LCB stain at 10× and 40×	End of incubation
Bacterial population density	CFU/mL	Total plate count on King's B medium	24 h after plating

Note: LCB = lactophenol cotton blue; CFU = colony-forming units.

Colony characteristics of *C. truncatum* and *P. fluorescens*, including color, texture, shape, surface, and growth rate, were observed macroscopically on the culture plates. For microscopic observation, a drop of lactophenol cotton blue (LCB) was placed on a clean glass slide in a laminar airflow cabinet. Mycelium of *C. truncatum* was collected with a sterile loop needle, placed in the LCB stain, covered with a coverslip, and examined under a light microscope (brand and model) at 10× and 40× objective magnifications to observe the hyphae and conidia. Samples were also taken from

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the interaction zone between the pathogen and the antagonist at the end of incubation to observe changes in hyphal morphology.

The viable population density of *P. fluorescens* was determined by the total plate count (TPC) method. One milliliter of the bacterial suspension was added to 9 mL of sterile distilled water in a test tube and homogenized on a shaker. Tenfold serial dilutions were prepared up to 10^{-10} by transferring 1 mL from each tube to the next. One milliliter of each dilution was spread on King's B medium with a sterile L-shaped glass spreader, and the plates were incubated for 24 h. Plates containing 30–300 colonies were counted, and the population density was calculated as CFU/mL = number of colonies × (1/dilution factor) × (1/volume plated).

Inhibition data at each observation time were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Differences among treatments were evaluated using Welch's ANOVA followed by the Games-Howell post hoc test, which does not assume equal variances among groups. Differences were considered significant at $p < 0.05$. Analyses were performed using software on a computer. Results are presented as mean ± standard deviation (SD). To describe the variability and temporal pattern of the response, the coefficient of variation (CV = SD/mean × 100%) and the change in inhibition between observation times were calculated from the treatment means. Morphological observations were analyzed descriptively.

RESULTS

All *P. fluorescens* concentrations inhibited the radial growth of *C. truncatum* toward the bacterial colony, whereas the pathogen in the control plates grew symmetrically (Figure 2). Inhibition in all three bacterial treatments was significantly higher than in the control at 24, 48, and 72 h (Table 3). However, the three concentrations did not differ significantly from each other at any observation time, so the hypothesis that inhibition would increase with concentration was not supported statistically.

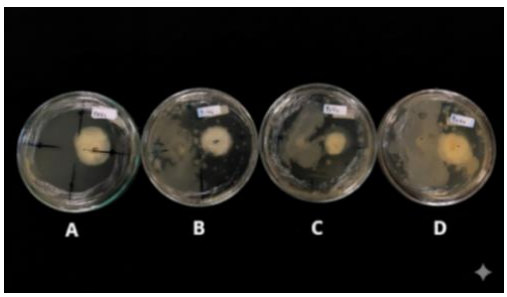

Figure 2. Inhibition of *C. truncatum* by *P. fluorescens* in dual culture: (A) control; (B) 10^7 CFU/mL; (C) 10^8 CFU/mL; (D) 10^9 CFU/mL

Table 3. Inhibition of *C. truncatum* by *P. fluorescens* at different concentrations and observation times

Treatment	24 h	48 h	72 h	Mean
Control (P0)	0.0 ± 0.00 a	0.0 ± 0.00 a	0.0 ± 0.00 a	0.00
P. fluorescens 10^7 CFU/mL (P1)	28.7 ± 15.37 b	16.7 ± 8.04 b	17.0 ± 8.64 b	20.80
P. fluorescens 10^8 CFU/mL (P2)	30.5 ± 12.05 b	23.9 ± 7.86 b	13.6 ± 3.12 b	22.66
P. fluorescens 10^9 CFU/mL (P3)	35.9 ± 11.33 b	20.0 ± 7.84 b	22.0 ± 7.64 b	25.96

Note: Values are inhibition (%) expressed as mean ± SD (n = 5). Means followed by the same letter in the same column are not significantly different according to the Games-Howell test ($p < 0.05$). Mean = average of the three observation times.

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Numerically, the mean inhibition over the three observation times increased with bacterial concentration, from 20.80% at 10^7 CFU/mL to 22.66% at 10^8 CFU/mL and 25.96% at 10^9 CFU/mL. The highest single value was recorded at 10^9 CFU/mL after 24 h (35.9%). The standard deviations were large relative to the means, particularly at 24 h, which indicates considerable variation among replicate plates.

Inhibition was highest at 24 h in all bacterial treatments and decreased thereafter (Table 4). Between 24 and 72 h, inhibition decreased by 11.7 percentage points at 10^7 CFU/mL, 16.9 percentage points at 10^8 CFU/mL, and 13.9 percentage points at 10^9 CFU/mL, which corresponds to relative reductions of 38.7–55.4%. Most of the decline at 10^7 and 10^9 CFU/mL occurred between 24 and 48 h, after which inhibition remained almost stable, whereas at 10^8 CFU/mL inhibition continued to decrease until 72 h. Variability was highest at 10^7 CFU/mL, with a CV above 50% at both 24 and 72 h.

When the three bacterial treatments were averaged, inhibition decreased from 31.7% at 24 h to 20.2% at 48 h and 17.5% at 72 h. The ranking of the concentrations also changed over time: 10^9 CFU/mL gave the highest inhibition at 24 and 72 h, whereas 10^8 CFU/mL gave the highest value at 48 h (23.9%) but the lowest at 72 h (13.6%). These shifts in ranking are consistent with the absence of significant differences among the concentrations.

Table 4. Change in inhibition of *C. truncatum* between observation times and coefficient of variation

Treatment	Δ 24–48 h (pp)	Δ 48–72 h (pp)	Δ 24–72 h (pp)	Relative change 24–72 h (%)	CV at 24 h (%)	CV at 72 h (%)
P1 (10^7 CFU/mL)	−12.0	+0.3	−11.7	−40.8	53.6	50.8
P2 (10^8 CFU/mL)	−6.6	−10.3	−16.9	−55.4	39.5	22.9
P3 (10^9 CFU/mL)	−15.9	+2.0	−13.9	−38.7	31.6	34.7
Mean of P1–P3	−11.5	−2.7	−14.2	−44.7	–	–

Note: Calculated from the treatment means and SD in Table 3; the control (0% at all times) is omitted. Δ = change in inhibition; pp = percentage points; CV = SD/mean × 100%.

The *C. truncatum* colonies were grayish-white, had a cotton-like texture, and grew rapidly. Microscopically, the pathogen had hyaline hyphae with blunt tips and cylindrical conidia. The *P. fluorescens* colonies were oval to round, with a smooth, milky-white to cream surface (Table 5). These characteristics are consistent with the descriptions of the two organisms used to identify them.

Table 5. Macroscopic and microscopic characteristics of *C. truncatum* and *P. fluorescens*

Characteristic	C. truncatum	P. fluorescens
Colony color	Grayish-white	Milky-white to cream
Colony texture and surface	Cotton-like	Smooth
Colony shape	–	Oval to round
Growth	Rapid	–
Microscopic features	Hyaline, septate, branched hyphae with blunt tips; cylindrical conidia	–
Interaction zone	Hyphal shrinkage and lysis	Colonies in contact with and spreading among hyphae

Note: – = not assessed. Microscopic observation with lactophenol cotton blue at 10× and 40× magnification.

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In the interaction zone, the bacterial colonies were in direct contact with and spread among the pathogen hyphae (Figure 3C). Hyphae close to the bacterial colonies showed morphological abnormalities, including shrinkage and lysis, whereas hyphae from the control plates were intact, septate, and branched (Figure 3A).

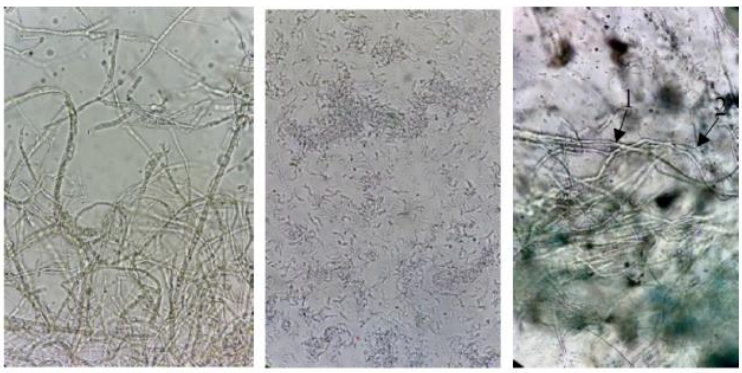

Figure 3. Microscopic observation: (A) hyphae of *C. truncatum*; (B) colony of *P. fluorescens*; (C) *P. fluorescens* colonies inhibiting *C. truncatum*, showing (1) abnormal pathogen hyphae and (2) bacterial colonies

The total plate count of the *P. fluorescens* suspension on King's B medium showed a viable population, confirming that the bacterium was viable and that the suspensions used in the assay corresponded to the intended concentration range.

DISCUSSION

This study showed that *P. fluorescens* inhibited the growth of *C. truncatum* in vitro at all tested concentrations. The level of inhibition was moderate, with mean values ranging from 20.80% to 25.96% over 72 h and a maximum of 35.9% at 24 h. These values are similar to the 36.08% inhibition of *Colletotrichum* sp. by *P. fluorescens* reported by Ramdan et al. (2021), who also observed large variation among replicates. Antagonistic pseudomonads typically show broad but strain-dependent antifungal activity, with the degree of antagonism varying among target fungi (Sokołowski et al., 2024). The present results therefore confirm that *P. fluorescens* is active against *C. truncatum* from edamame but also indicate that the isolate tested has only moderate antagonistic potential when used alone under dual-culture conditions.

The way inhibition was measured should also be considered when interpreting these values. The formula compares the radius of the pathogen colony growing toward the antagonist with that growing away from it on the same plate, so each plate serves as its own internal reference. This approach corrects for differences in the pathogen's overall growth across plates, but it is sensitive to measurement error when colonies are small. At 24 h, the pathogen colony had grown only a short distance from the inoculation plug, and a 1 mm difference between the two radii could change the calculated inhibition by several percentage points. This probably explains why the standard deviations were largest at 24 h and why the highest inhibition values were recorded at the first observation. As the colonies expanded, the two radii increased, and the estimates became more stable, as shown by the lower CV values at 72 h for 10^8 CFU/mL. Measuring colony area by image analysis or extending the observation period until the pathogen on the control plates covers the dish would yield more precise estimates of inhibition in future experiments.

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Contrary to our hypothesis, increasing the concentration from 10^7 to 10^9 CFU/mL did not significantly increase inhibition, although the mean values showed a consistent upward trend. In other antagonistic systems, inhibition has been reported to depend on antagonist dose (Bao et al., 2023). In Pseudomonas, the production of phenazines and other antifungal metabolites is regulated by quorum sensing and increases with cell density (Xia et al., 2024). In a dual culture assay, however, the bacterium is applied as a small inoculum on a nutrient-rich medium, where it multiplies rapidly, and the local cell density soon reaches levels at which quorum-sensing-regulated genes are expressed, regardless of the initial concentration. The initial difference of two orders of magnitude may therefore have been reduced during the first hours of incubation. In addition, the standard deviations were large, with CV values of 22.9–53.6%, and only five replicates were used. Under such conditions, the statistical power to detect differences of a few percentage points between concentrations is low. The Games-Howell test, which is appropriate for unequal variances, is also relatively conservative. The absence of significant differences should therefore be interpreted as a lack of evidence for a concentration effect within the tested range rather than as proof that concentration has no effect.

Inhibition was highest at 24 h and declined at 48 and 72 h. This pattern suggests that the isolate's antagonistic effect was primarily fungistatic and that *C. truncatum*, a fast-growing fungus, gradually recovered and grew toward the bacterial colony. Several factors may contribute to this decline. Antifungal metabolites diffuse into the medium and form a concentration gradient around the bacterial colony; as the fungal colony expands, a larger portion of its margin is at metabolite concentrations that are low. Nutrients in the medium are also depleted over time, which may reduce the bacterium's metabolic activity. Similar loss of activity is known from inoculation studies, in which the population of introduced bacteria declines after application unless the inoculation is repeated (Papin et al., 2024). For practical application, this implies that a single application of *P. fluorescens* may provide only short-term protection, and that repeated applications or formulations that support the bacterium's survival on the plant surface should be evaluated.

Competition for space and nutrients is likely to be one of the main mechanisms of inhibition in this study. On NA medium, the bacterium grew rapidly and occupied the area between the inoculation points, which restricted the space available for fungal growth. Pseudomonads are motile via polar flagella, which enable them to spread and colonize surfaces effectively (Bouteiller et al., 2021). The physical structure of the habitat also influences interactions between bacteria and fungi: in homogeneous environments, such as the agar surface of a Petri dish, fungal hyphae do not benefit from the spatial heterogeneity that allows them to bypass competitors in soil (Arellano-Caicedo et al., 2021). Competition for iron is another possible mechanism. Fluorescent pseudomonads produce pyoverdine, a siderophore with a very high affinity for ferric iron, and under iron-limited conditions, pyoverdine is one of the most important antifungal molecules of *Pseudomonas* (Sass et al., 2021). Siderophore production is one of the best-documented traits of *P. fluorescens* (Muthiah et al., 2023; Rusendra et al., 2024).

Antibiosis may also have contributed to the inhibition. Plant-beneficial pseudomonads produce phenazines, which are redox-active compounds with broad-spectrum antifungal activity (Biessy and Filion, 2018), and phloroglucinol derivatives such as 2,4-diacetylphloroglucinol, which disrupt the membranes and growth of many fungal pathogens (Biessy and Filion, 2021). Other compounds, including pyrrolnitrin, diketopiperazines, hydrogen cyanide, and volatile organic compounds, have been identified in antagonistic *Pseudomonas* strains (Sokołowski et al., 2024; Gusnadi et al., 2023). The production of these metabolites differs among strains, which may explain why the inhibition reported for *P. fluorescens* against *Colletotrichum* varies widely between studies (Mehmood et al.,

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2023). Because the present study did not include culture filtrate or volatile compound assays, the relative importance of competition and antibiosis could not be separated.

Microscopic observation provided further evidence of antagonism. Hyphae of *C. truncatum* in the interaction zone showed shrinkage and lysis, whereas control hyphae were intact. Comparable mycelial malformation of *Colletotrichum* has been reported after exposure to antagonistic phylloplane bacteria (Widiantini et al., 2024). Such abnormalities are consistent with the action of membrane-active antibiotics and cell-wall-degrading enzymes, such as chitinases and glucanases, produced by many biocontrol pseudomonads (Nerek and Sokołowska, 2022; Guzmán-Guzmán and Santoyo, 2022). Damage to the hyphal tip may reduce the pathogen's ability to infect host tissue, even if growth is not completely halted. Ultrastructural studies and enzyme assays are needed to confirm which mechanism is responsible for the observed damage.

From a practical perspective, these results support further evaluation of *P. fluorescens* as a component of anthracnose management in edamame. Anthracnose can cause substantial yield losses in soybean (Dias et al., 2019; Boufleur et al., 2021), and biological control agents that reduce early pathogen growth may help delay the development of epidemics. *P. fluorescens* also has plant growth-promoting traits (Muthiah et al., 2023), and *Pseudomonas* is naturally associated with legume roots and nodules (Febriani et al., 2023; Hnini and Aurag, 2024), which may favor its establishment on edamame. Because the three concentrations were statistically equivalent, the lower concentrations of 10^7–10^8 CFU/mL may be sufficient for further testing, thereby reducing the cost of producing the biological agent. Combining *P. fluorescens* with other antagonists or with reduced fungicide doses may also improve and stabilize the level of control (Okwisan et al., 2023).

This study has several limitations. Only one isolate of each organism was tested, and neither was identified by molecular methods. The dual culture assay was performed on NA medium, which favors bacterial growth and may not reflect conditions on the plant surface. Observations were limited to 72 h, so the long-term outcome of the interaction is unknown, and the variability among replicates was high. Future studies should include molecular identification of the isolates, more replicates, comparisons across different media, culture filtrate and volatile compound assays to elucidate the mechanisms involved, and greenhouse and field experiments to determine whether the in vitro antagonism translates into reduced anthracnose severity and improved pod quality in edamame.

CONCLUSION

Pseudomonas fluorescens at 10^7, 10^8, and 10^9 CFU/mL significantly inhibited the in vitro growth of *Colletotrichum truncatum* compared with the control, but the three concentrations did not differ significantly from each other. The highest mean inhibition was obtained at 10^9 CFU/mL (25.96%), followed by 10^8 CFU/mL (22.66%) and 10^7 CFU/mL (20.80%). Inhibition was greatest at 24 h and decreased by 72 h, and the bacterium caused shrinkage and lysis of the pathogen hyphae in the interaction zone. These results indicate that *P. fluorescens* has moderate antagonistic activity against *C. truncatum*; because higher concentrations did not significantly improve inhibition, 10^7–10^8 CFU/mL can be considered for further greenhouse and field evaluation of edamame anthracnose control.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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