

# Preliminary screening of rhizosphere bacteria in shallot plants grown in organic field as antagonists against *Fusarium oxysporum* f.sp. *cepae*

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**Abstract.** Fusarium wilt disease, caused by *Fusarium oxysporum* f. sp. *cepae* (*Focc*), is a devastating disease affecting shallot plants. This study aimed to screen rhizosphere bacteria isolated from healthy shallot plants cultivated in organic field and to evaluate their antagonistic potential against *Focc*. A total of 29 rhizosphere bacterial isolates were found from healthy shallot plants in Jember, East Java, Indonesia. These isolates exhibited considerable variation in morphological characteristics, including colony color, shape, and opacity. The bacterial density in the samples generally ranged from  $10^7$  to  $10^9$  CFU/ml, with colony numbers differing among isolates. The predominant colony colors were white and yellow, with morphological forms ranging from circular to irregular and serrated. Among these, thirteen isolates (RB1, RG1, RH1, RI1, RJ1, RF2, RA3, RB3, GC3, GD3, GRA4, GRC4, and GRE4) demonstrated strong antagonistic potential against *Focc*, with inhibition percentages ranging from 56.60 to 68.79%. These findings indicate that rhizosphere bacteria from shallot plants possess antagonistic properties and hold potential as biocontrol candidates against Fusarium wilt. However, further in vitro antagonistic assays and molecular identification are required to confirm the bacterial species involved and to determine their efficacy in suppressing Fusarium wilt while promoting shallot growth.

## 1 Introduction

Shallot (*Allium cepa* L. var. *ascalonicum*) is an economically significant *Allium* crop, widely cultivated for both culinary and medicinal uses. However, its productivity is severely constrained by Fusarium wilt, a soil-borne disease caused by pathogens, particularly *Fusarium oxysporum* f. sp. *cepae* (*Focc*), which causes basal rot, wilting, or 'moler' disease in shallow soils [1]. In the management of Fusarium wilt, farmers frequently rely on chemical pesticides; however, excessive application has been shown to cause detrimental ecological effects, including the elimination of beneficial soil microorganisms, disruption of non-target species, loss of plant biodiversity, and long-term ecosystem degradation [1]. Continuous reliance on fungicides is

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not recommended, as it contributes to environmental pollution of soil and water resources and may lead to fungicide resistance. Moreover, chemical control is often economically unsustainable due to the high cost and repeated application requirements. In Indonesia, the use of chemical pesticides remains high, raising concerns regarding increased production costs, mortality of non-target organisms, and the overall decline in environmental quality [2].

Recent studies have increasingly focused on the use of biological control agents as sustainable alternatives to chemical pesticides for managing *Fusarium oxysporum* f. sp. *cepae* (*Focc*) infections. Several reports have demonstrated that bacterial isolates obtained from shallot tissues or associated environments possess antagonistic activity against *Focc*, both *in vitro* and *in planta*. For instance, isolated bacterial strains from shallot plants and reported that *Bacillus subtilis* (AB3, TB2) and *Pseudomonas nitroreducens* (UB1) inhibited *Focc* mycelial growth by 35–47% under *in vitro* condition. Similarly, researchers obtained endophytic bacterial isolates from non-*Allium* hosts such as ferns, mangroves, and peppers, which also exhibited inhibitory effects against *Focc* in culture and plant protection assays [3]. These findings highlight the potential of native bacterial communities as promising biocontrol candidates for sustainable shallot disease management

Organic farming systems reduced synthetic chemical use and higher soil organic matter, have been found to promote more beneficial microbiomes in the rhizosphere, which can contribute to disease suppression. Long-term organic farming was shown to increase the abundance of *Bacillus* antagonists and improve suppression of fungal and oomycete pathogens under greenhouse conditions (e.g. in pepper-*Phytophthora capsici* systems) when compared to conventional agriculture [4]. Also, combining organic manure with specific bacterial inoculants has led to stable suppression of *Fusarium* wilt in tomato via direct antagonism and reshaping of rhizosphere bacterial communities [5].

Despite these advances, important gaps remain. First, studies specifically targeting rhizosphere bacteria in shallots under organic field conditions are scarce. Most work has been done *in vitro* or in controlled environments; few isolates are tested from organic systems where microbial diversity and functionality may differ significantly. Second, there is limited information on which bacterial taxa are both effective antagonists *and* well adapted to field conditions in shallot production. Third, many studies assess only inhibition *in vitro*, without subsequent evaluation of consistent suppression under field or semi-field conditions. Therefore, this study aims to address these gaps by Preliminarily screening rhizosphere bacteria isolated from shallot plants grown under organic field conditions for antagonistic activity against *Focc* pathogen. Specifically, the objectives are to isolate rhizosphere bacteria from organic shallot fields, test their *in vitro* antifungal activity against *Focc*, and identify promising isolates (by molecular methods) for further greenhouse or field efficacy trials. The outcome is expected to contribute to the development of effective multi-purpose biocontrol agents adapted to the ecological conditions of shallot cultivation in organic systems.

## 2 Materials and Methods

### 2.1. Rhizosphere sampling

The study was conducted in shallot cultivation fields located in Jember, East Java, Indonesia. The research site was located between the coordinates -8.157136, 113.725722 and -8.156591, 113.725476. The field was cultivated with healthy shallot plants and treated with compost, along with the application of several biological agents such as *Trichoderma* and organic pesticides. These practices were expected to influence the abundance and diversity of bacterial isolates, particularly under an organic farming system. Rhizosphere-samples of shallots were collected from healthy plants and soils across four different fields. For systematic sampling, three sampling points were taken from each plant. The bacterial

isolation procedure followed the method described by Gams [6]. Soil samples were collected at a depth of approximately 20 cm near the root zone, combined, and placed in sterile plastic bags for further processing.

## 2.2 Isolation bacteria from rizosphere shallot plant

Bacterial isolation was performed using the dilution plate technique [7]. A homogenized soil sample (1 g) was suspended in 10 mL of sterile distilled water in an Erlenmeyer flask. From this suspension, serial dilutions ranging from  $10^{-2}$  to  $10^{-9}$  were prepared. Subsequently, 100  $\mu$ L of the bacterial suspension from dilutions of  $10^{-7}$  to  $10^{-12}$  was spread onto nutrient agar (NA) plates using the spread plate method. The inoculum was evenly distributed with an L-shaped glass rod until completely absorbed, and the plates were incubated at room temperature for 48 hours. Distinct bacterial colonies that developed were then purified [8]

## 2.3 Bacterial morphology characterization

The morphological characterization of bacterial colonies was conducted as a preliminary step in the identification process. This characterization involved observing and recording several phenotypic traits of the colonies, including their overall shape, margin structure, coloration, and surface appearance. These morphological features provided the basis for the initial differentiation and tentative identification of the bacterial isolates [9,10].

## 2.4 Screening for antagonistic activity

Antagonistic potential of bacterial isolates against *Fusarium oxysporum* f. sp. *cepae* (*Focc*) was assessed using the dual culture assay. A 5-mm agar plug of actively growing *Focc* culture was placed at the center of Potato Dextrose Agar (PDA) plates, and bacterial isolates were streaked 3 cm away from the fungal plug [11,12]. Plates inoculated with *Focc* alone served as controls. Cultures were incubated at 27°C for 7 days. Antagonistic activity was evaluated by measuring the inhibition zone and percentage of mycelial growth suppression relative to the control, the inhibition strong is more than 50% [10]

# 3 Results and Discussion

## 3.1 Bacterial Abundance in the Rhizosphere of Shallot Plants

The exploration results revealed the diversity and abundance of rhizosphere bacteria associated with shallot plants, with each isolate designated by a different code. The abundance of rhizosphere bacteria is presented in Table 1. Considerable variation in bacterial abundance was observed among treatments and isolate codes, suggesting differences in the colonization capacity of individual isolates within plant tissues. Isolates with higher population densities are considered to have strong potential as biological control agents, since a greater cell density enhances competition for space and nutrients with pathogenic microbes, while simultaneously promoting the production of bioactive metabolites such as chitinase enzymes and natural antibiotic compounds that can suppress pathogen development. Increased cell density is also known to activate genes associated with antimicrobial metabolite biosynthesis, particularly during the stationary growth phase, when production of enzymes such as chitinase typically occurs [13]. This finding is consistent with the study by Widowati [14], who successfully isolated 75 shallot-associated bacterial strains and reported that most of them were capable of producing antibiotics and hydrolytic enzymes, which not only promote plant growth but also enhance resistance against pathogens.

Based on macroscopic morphological characterization, 29 rhizosphere bacterial isolates with distinct colony morphologies were obtained. These isolates were recovered through serial dilution (dilution plate) techniques. Colony morphology was observed in terms of

shape, color, and opacity, as summarized in Table 2. Morphological characterization revealed colony diversity in color (white, yellow, green, orange, red), shape (circular, irregular, serrated), and opacity (transparent, opaque, glossy), indicating physiological differences among isolates. Such variation in colony morphology reflects a rich diversity of the rhizosphere bacteria community and suggests both physiological and genetic heterogeneity. Previous study successfully isolated endophytic bacteria from shallots with diverse shapes, elevations, and colors, highlighting the microbial diversity within shallot tissues [15]. As reported previously [16], morphological diversity often mirrors the complexity of endophytic microbial communities in shallot species and may correlate with differences in metabolic functions, including the production of secondary metabolites that act as natural antibiotics. Biologically, a high population of endophytic bacteria is essential, as it enhances competitiveness against pathogenic microbes through nutrient competition and colonization space.

**Table 1.** Abundance of rhizosphere bacteria isolated from healthy shallot

Number	Isolate code	Abundance (CFU/ g)
1	RA1	141x10 <sup>7</sup>
2	RB1	187x10 <sup>9</sup>
3	RC1	140x10 <sup>6</sup>
4	RD1	100x10 <sup>6</sup>
5	RE1	200x10 <sup>6</sup>
6	RF1	110x10 <sup>7</sup>
7	RG1	100x10 <sup>7</sup>
8	RH1	100x10 <sup>7</sup>
9	RI1	200x10 <sup>6</sup>
10	RJ1	100x10 <sup>7</sup>
11	RA2	273x10 <sup>6</sup>
12	RB2	50x10 <sup>8</sup>
13	RC2	155x10 <sup>7</sup>
14	RD2	177x10 <sup>7</sup>
15	RE2	50x10 <sup>7</sup>
16	RF2	20x10 <sup>7</sup>
17	GA3	76x10 <sup>9</sup>
18	GB3	100x10 <sup>5</sup>
19	GC3	128x10 <sup>9</sup>
20	GD3	93x10 <sup>8</sup>
21	GE3	100x10 <sup>7</sup>
22	GF3	98x10 <sup>8</sup>
23	GRA3	50x10 <sup>7</sup>
24	GRA4	55x10 <sup>6</sup>
25	GRB4	40x10 <sup>6</sup>
26	GRC4	180x10 <sup>6</sup>
27	GRD4	200x10 <sup>6</sup>
28	GRE4	50x10 <sup>6</sup>
29	GRF4	200x10 <sup>5</sup>

**Table 2.** Macroscopic morphology of rhizosphere bacteria isolated from shallot plants

Isolate Code	Colony Color	Colony Shape	Opacity
RA1	Green	Circular	Shiny
RB1	Green	Irregular	Transparent
RC1	Red	Irregular	Opaque
RD1	Orange	Circular	Opaque
RE1	White	Circular	Opaque
RF1	White	Irregular	Opaque
RG1	White	Circular	Transparent
RH1	White	Circular	Shiny
RI1	Yellow	Irregular	Transparent
RJ1	Orange	Circular	Transparent
RA2	Yellow with green edge	Circular	Opaque
RB2	White	Circular	Opaque
RC2	Yellow with white edge	Circular	Opaque
RD2	White	Layered circular	Transparent
RE2	White	Irregular	Opaque
RF2	White	Irregular	Transparent
GA3	White	Circular	Transparent
GB3	White	Circular	Shiny
GC3	White	Irregular	Opaque
GD3	Yellow	Irregular	Transparent
GE3	Yellow	Serrated	Transparent
GF3	White	Serrated	Transparent
GRA3	White	Serrated	Opaque
GRA4	White	Circular	Shiny
GRB4	White	Layered circular	Transparent
GRC4	Yellow	Circular	Shiny
GRD4	White	Irregular	Opaque
GRE4	Yellow	Serrated	Transparent
GRF4	White with yellow edge	Circular	Opaque

### 3.2 Screening of Antagonistic Bacteria from Shallot Rhizosphere

The antagonistic screening assay demonstrated that all twenty-nine rhizosphere bacterial isolates from shallot were capable for inhibiting *Focc* growth to varying degrees after seven days of incubation (Table 3). Among the isolates, several showed moderate inhibition, while others exhibited stronger suppression of mycelial growth. Notably, isolate GRA4 displayed the highest antagonistic activity, indicating its strong potential as a biocontrol candidate. In contrast, isolate HD2 showed relatively weak inhibition, suggesting lower antifungal capacity. These findings highlight the variability of antagonistic performance among rhizosphere bacteria, which may be attributed to differences in their underlying mechanisms, such as enzyme production, antibiotic secretion, or competition for space and nutrients [17].

**Table 3.** Antagonistic screening activity of rhizosphere bacterial against *Focc* pathogen at day 7

Number	Isolate code	Inhibition percentage (%)	Number	Isolate code	Inhibition percentage (%)
1	RA1	51.06	16	RF2	56.60
2	RB1	59.56	17	GA3	63.99
3	RC1	54.94	18	GB3	67.13
4	RD1	53.46	19	GC3	60.30

Number	Isolate code	Inhibition percentage (%)	Number	Isolate code	Inhibition percentage (%)
5	RE1	56.23	20	GD3	62.51
6	RF1	50.51	21	GE3	55.86
7	RG1	61.77	22	GF3	54.39
8	RH1	64.36	23	GRA3	58.26
9	RI1	65.65	24	GRA4	68.79
10	RJ1	56.97	25	GRB4	51.80
11	RA2	48.66	26	GRC4	59.93
12	RB2	44.60	27	GRD4	47.74
13	RC2	47.68	28	GRE4	65.28
14	RD2	43.49	29	GRF4	54.57
15	RE2	52.91	30	Control	0

Antagonistic screening assays revealed that among 29 rhizosphere bacterial isolates from shallot, inhibition of *Focc* mycelial growth ranged from 43.49% to 68.79%, with isolate A4 exhibiting the highest activity (68.79%). Isolates with inhibition rates exceeding 60% are regarded as having a sufficiently strong biocontrol capability. The ability of rhizosphere bacteria to suppress *Focc* is associated with antifungal mechanisms such as lysis, antibiosis, hyperparasitism, and competition [18]. Chitinase production, reported as a key physiological trait of shallot rhizobacteria [15], further supports their biocontrol potential. Previous research on rhizobacteria has produced isolates with 73,02% inhibition capability and several studies have also reported rhizobacterial isolates that produce secondary metabolites [19]. In another isolates, chilli-derived rhizobacterial isolates Iso32 and Iso24 showed pronounced antagonism toward *Fusarium oxysporum* f. sp. *capsici*, inhibiting pathogen growth by 73.3% and 71.5% in vitro [20].

The variation in inhibition levels indicates differences in antagonistic mechanisms, including hydrolytic enzyme activity, antibiotic and siderophore production, and competition for space and nutrients. Nutrient competition generally increases with higher densities of antagonistic cells and represents a major mechanism in many yeast- and bacteria-based biocontrol systems [21–23]. Previous findings similarly demonstrated that several shallot rhizosphere isolates achieved inhibition rates above 60%, underscoring the crucial role of rhizobacteria in suppressing soil-borne pathogens [18]. A large-scale field evaluation of antagonistic rhizobacterial formulations in shallot production reported 73–95% inhibition of *Fusarium* wilt under farmer-managed conditions, highlighting that specific isolates or consortia can deliver disease suppression above 60% in real-world settings [24]. In another shallot-based investigation, rhizospheric *Bacillus* spp. exhibited antagonistic activity against *F. oxysporum*, achieving approximately 30% growth inhibition in vitro through antibiosis and hyphal swelling mechanisms [25]. Collectively, these results highlight that certain rhizobacteria function not only as root colonizers but also as promising biocontrol agents. This supports earlier findings on the significant role of rhizobacteria as biocontrol agents against pathogens such as *F. oxysporum* f.sp. *lycopersici* dan *Colletotricum capsici* [26,27].

## 4 Conclusion

This study successfully isolated and characterized rhizosphere bacteria associated in shallot plants. Morphological characterization revealed considerable diversity in colony color, shape, and opacity, reflecting both physiological and genetic variation among isolates.

Antagonistic screening assays demonstrated that several isolates effectively inhibited the growth of *Focc*, with inhibition rates ranging from 43.49% to 68.79%. The findings highlight the potential of shallot-associated bacteria not only as colonizers but also as promising sources of hydrolytic enzymes, bioactive metabolites, and natural antagonists against soil-borne pathogens.

## Acknowledgment

The author would like to thank P3M and Politeknik Negeri Jember for funding this research from the Research on Non-Tax State Revenue Sources (PNBP) under Contract Number 0893/PL17.4/PG/2025. The dissemination of this research was recognized with the Best Presenter award at the 8th International Conference on Food and Agriculture (ICoFA) 2025.

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