Proceedings of the International Conference on Agricultural Strategy for Sustainability (ICASS)
October 22–23, 2025 – Pekanbaru, Indonesia
Volume 1, Number 1 (2026): 1-6

The Potential of Peat Soil Bacteria from Teluk Belitung Village as Antagonistic Agents against *Fusarium oxysporum*

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Abstract. Utilization of bacteria as biological control agents is one of the alternatives to reduce the use of synthetic pesticides. Peat soil is known to be a habitat for bacteria with potential as biological agents in controlling phytopathogens such as *Fusarium oxysporum*. This study aimed to evaluate the potential of peat soil bacteria isolated from Teluk Belitung Village as antagonistic agents against *F. oxysporum*. A qualitative descriptive method was employed. Isolation was carried out using the spread plate technique. The obtained isolates were examined for colony and microscopic characteristics, catalase activity, cells number, and antagonistic activity against *F. oxysporum*. Five bacterial isolates (IS1, IS2, IS3, IS4, and IS5) were successfully obtained. The isolates were rod-shaped, Gram-negative, and exhibited catalase activity. Isolate cells number between 0.02 to 7.20 x 109 CFU.g-1. All isolates demonstrated antagonistic activity against *F. oxysporum*, with growth inhibition rates ranging from 18.75 to 77.50%. Based on growth inhibition, isolates IS1, IS2, and IS3 showed the greatest potential as antagonistic agents against *F. oxysporum*.

Keywords: Antagonistic bacteria, Biological control, *Fusarium oxysporum,* Peat soil bacteria, Plant pathogenic fungi.

1. Introduction

Peat soil is a distinctive ecosystem commonly found in tropical regions, including Indonesia. Unlike mineral soils, peat is characterized by its high organic matter content, relatively low pH, and predominantly anaerobic conditions [1], [2]. These unique properties make peatlands a favorable habitat for diverse microorganisms [3], particularly bacteria [4].Such microbial diversity is believed to hold great potential for various applications, especially in the development of sustainable agriculture [5], [6].

One of the major challenges in agriculture is the presence of soil-borne pathogens, among which *Fusarium oxysporum* is of particular concern. This pathogen is widely recognized as the causal agent of Fusarium wilt in many cultivated crops [7], [8]. Its infection can cause severe yield losses due to its ability to survive in soil for extended periods through the formation of chlamydospores [9]. To date, the control of *F. oxysporum* has largely relied on synthetic pesticides, which in the long term pose detrimental effects on the environment, human health, and soil ecosystem sustainability [8].

As an alternative, the use of microbial-based biocontrol agents has gained increasing attention. Several bacterial species are known to possess antagonistic properties through mechanisms such as competition for space and nutrients, production of antimicrobial metabolites, and induction of systemic resistance in plants. The application of antagonistic

bacteria offers the dual advantage of suppressing plant pathogens while promoting environmentally friendly and sustainable farming systems. Given its microbial richness, peat soil is considered a promising source of novel bacterial candidates with potential biocontrol activity [10], [11].

In this context, the present study was conducted to evaluate the potential of bacterial isolates from peat soil in Teluk Belitung Village as antagonistic agents against *F. oxysporum*. The findings are expected to provide preliminary insights into the utilization of local microbial resources as alternative biocontrol agents, while also contributing to the growing body of research on the role of peatland microbial diversity in sustainable agriculture in Indonesia.

2. Research Methods

2.1 Soil sampling

Soil samples were collected using a purposive sampling technique [12]. The peat soil was obtained from a rubber plantation located in Teluk Belitung Village, Kepulauan Meranti Regency, Riau Province, Indonesia. The peat soil is sapric type. Peat soil was taken at five points with a depth of 0 to 20 cm. The weight of the soil taken at each point was 10 g and then composited. The peat soil was stored in a cool box. Samples were then transported to the laboratory for bacterial isolation [13].

2.2 Isolation, colony characterization, and enumeration of bacteria

Bacterial isolation was carried out on sterile nutrient agar (NA) medium. Approximately 10 g of peat soil was suspended in 90 mL of sterile 0.85% NaCl solution within an Erlenmeyer flask, then subjected to serial dilutions up to 10^{-8}. Each dilution step was homogenized using a vortex mixer [14]. Isolation was performed by the pour-plate technique from dilutions ranging between 10^{-6} and 10^{-8}, following the procedures outlined by [15] and [16]. The culture plates were incubated at 27°C for 24 hours.

Distinct morphological colonies were treated as separate bacterial isolates [15], [16]. The isolate population was determined using the following formula:

$$\text{Cells number } \left(\text{CFU g}^{-1}\right) = \text{Number of colonies x Dilution factor}$$

2.3 Purification of isolates

Bacterial isolates were purified on nutrient agar using the four-quadrant streak technique under aseptic conditions. The inoculated plates were incubated at 27 °C for 24–48 hours. Distinct colonies were subsequently transferred to sterile agar slants and incubated under identical conditions. The resulting pure isolates were further examined through microscopic observations, catalase activity assays, and tests for antagonistic potential. [17], [18].

2.4 Microscopic characterization and catalase activity

Microscopic characterization included determination of cell morphology and Gram staining. Gram staining was performed by smearing a loopful of bacterial culture onto a sterile glass slide pre-treated with sterile distilled water. The smear was heat-fixed by passing it briefly over a flame until dry, followed by sequential staining: crystal violet for 1 min, rinse with distilled water, Lugol's iodine for 1 min, rinse, safranin for 30 s, rinse, and air-dry [19]. Slides were examined under a light microscope at 1,000× magnification.

Catalase activity was assessed by placing a drop of 3% hydrogen peroxide (H_2O_2) on a sterile glass slide, followed by the addition of bacterial biomass using a sterile toothpick under aseptic conditions. The formation of oxygen bubbles indicated positive catalase activity [20].

2.5 Antagonistic activity assays

Antagonistic activity was assessed using a modified Dual Culture Assay as described by [21]. One milliliter of bacterial suspension (10^6 cells·mL^{-1}) was added to sterile Petri dishes for the

treatment group, while control plates received no bacterial inoculum. Both treatment and control plates were overlaid with sterile potato dextrose agar (PDA) at 50°C and homogenized. Once solidified, a 10 mm diameter plug of *F. oxysporum*. was inoculated at the center of each plate. Plates were incubated at 27°C for 7 days.

On the final day, the diameter of *F. oxysporum* colonies was measured by drawing vertical and horizontal lines intersecting at the colony center on the bottom of each Petri dish. The percentage of growth inhibition was calculated as:

$$\text{Growth inhibiton (\%)} = \frac{\text{Control diameter -Treatment diameter}}{\text{Control diameter}} \text{ x } 100\%$$

3. Results and Discussion

3.1 Bacterial isolate characteristics, catalase activity and cells number

Five bacterial isolates were obtained, each displaying diverse colony morphologies. Isolate IS1 exhibited a milky-white color with a filamentous form, whereas IS2 and IS3 both showed cream-colored, irregular colonies but differed in margin and elevation. IS4 also presented a milky-white color with an irregular form, while IS5 appeared more uniform with a circular shape and entire margin. Microscopic observations revealed that all isolates were rod-shaped (bacilli) and classified as Gram-negative bacteria. In addition, all isolates tested positive for catalase activity. The cells number ranged from 0.02×10^9 to 7.20×10^9 CFU g^{-1}(Table 1).

Table 1. Bacterial isolate characteristics, catalase activity and cells number

Parameter		Isolates				
		IS1	IS2	IS3	IS4	IS5
Colony Characteristics	Colour	Milky White	Cream	Cream	Milky White	Milky White
	Form	Filamentous	Irregular	Irregular	Irregular	Circular
	Margin	Filamentous	Undulate	Lobate	Undulate	Entire
	Elevation	Umbonate	Flat	Umbonate	Convex	Pulvinate
Microscopic Characteristics	Cell Shape	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
	Gram	Negative	Negative	Negative	Negative	Negative
Catalase Activity		Positive	Positive	Positive	Positive	Positive
Cells Number (CFU.g^{-1})		0.03 x 10^9	0.02 x 10^9	7.20 x 10^9	0.05 x 10^9	0.26 x 10^9

Variations in colony characteristics, including color, form, margin, and elevation, indicated phenotypic diversity among bacteria adapted to peat soil environments. Milky-white colonies, observed in IS1, IS4, and IS5, are generally associated with non-pigmented bacterial genera [22], whereas the cream-colored colonies of IS2 and IS3 may reflect differences in secondary metabolite composition [23]. Colony forms ranging from irregular and circular to filamentous suggest variations in growth patterns, likely influenced by nutrient content and the unique physical properties of peat soils [24].

The presence of Gram-negative bacteria suggests their dominance in acidic soil ecosystems such as peatlands, as their cell wall structure, with lipopolysaccharide layers, provides resistance to anaerobic conditions and pH fluctuations [25,26]. The ability to produce catalase further enhances their resistance to oxidative stress, equipping them with enzymatic defense mechanisms for survival in dynamic environments [26].

Cell density (CFU g^{-1}) varied considerably among isolates. IS3 exhibited the highest population, 7.20×10^9 CFU g^{-1}, indicating superior growth capacity and adaptation compared to the others. The abundance of IS3 may reflect its efficiency in utilizing organic substrates present in peat soil [27]. In contrast, IS2 showed the lowest population (0.02×10^9 CFU g^{-1}), possibly due to metabolic limitations or greater sensitivity to environmental conditions [28]. Such variation in bacterial populations aligns with previous studies reporting differences in growth rates and dominance within peat soil microbial communities [29].

Overall, these isolation results demonstrate that peat soils serve as a potential habitat for Gram-negative bacteria with strong catalase activity, while also revealing dominant isolates such

as IS3 that warrant further exploration as potential biocontrol agents or sources of functional metabolites. These findings highlight the importance of microbial biodiversity exploration in peat soils, not only for understanding microbial ecology but also for applications in agriculture and environmental biotechnology.

3.2 Antagonistic activity

The five isolates exhibited varying degrees of antagonistic activity against *Fusarium oxysporum*, with inhibition zones ranging from 18–77%. IS1 and IS3 showed the strongest inhibition (77%), IS2 displayed 69%, while IS4 demonstrated the lowest inhibition (18%). The antagonistic activity of each isolate against *F. oxysporum* is presented in Figure 1.

Isolates IS1, IS2, and IS3 demonstrated potential as effective biocontrol agents. Their strong inhibitory effects are likely related to the production of antifungal secondary metabolites such as antibiotics, siderophores, lytic enzymes (chitinase, β-1,3-glucanase), or volatile compounds [30]–[32]. These mechanisms are known to degrade fungal cell walls, inhibit hyphal division, and suppress pathogen colonization on host plants.

In contrast, IS5 exhibited moderate inhibition (±50%), possibly due to limited antifungal metabolite production or reduced effectiveness of its antagonistic mechanisms. IS4 showed the weakest inhibition (±18%), indicating a relatively low potential as a biocontrol agent [33]. Such variations highlight that not all bacterial isolates possess equal biocontrol capacities, as their effectiveness is strongly influenced by physiological and genetic traits [34], [35].

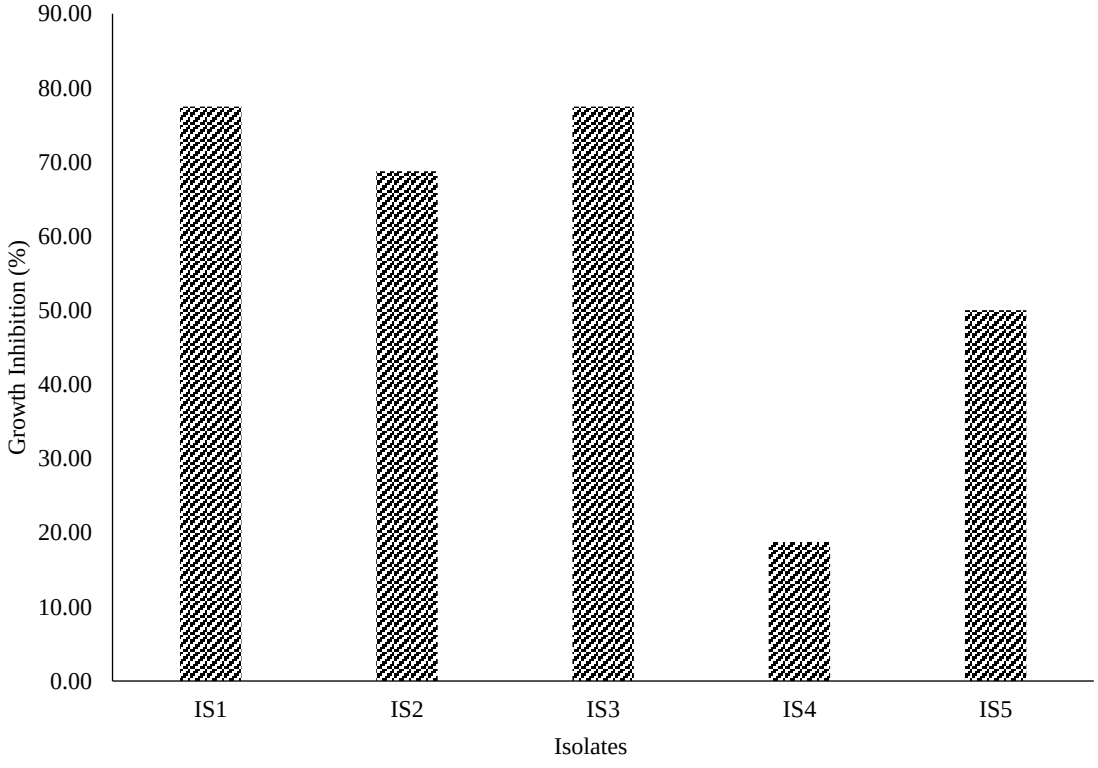

Figure 1. Antagonistic activity of bacterial isolates against *F. oxysporum*

In general, these findings support the view that soil bacteria represent promising sources of candidate biocontrol agents for suppressing soilborne pathogens. The use of highly inhibitory isolates (IS1, IS2, and IS3) could serve as an alternative strategy for the biological control of *F. oxysporum*, a major pathogen of various horticultural crops. This approach not only has the

potential to reduce crop yield losses but also to minimize dependence on synthetic fungicides, which pose risks to both the environment and human health [36], [37].

However, in vitro assays such as this represent only a preliminary step in screening biocontrol agents. Therefore, further studies are required to identify the potential isolates at the molecular level, assess the stability of their antagonistic activity under different environmental conditions, and evaluate their effectiveness through greenhouse and field trials [38].

4. Conclusions

This study successfully isolated five bacterial isolates (IS1–IS5) from the peat soil of Teluk Belitung Village, all of which were Gram-negative, rod-shaped, and catalase-positive, with population densities ranging from 0.02 to 7.20 × 10^9 CFU g^{-1}. In vitro antagonistic assays demonstrated that all isolates inhibited the growth of *Fusarium oxysporum*, with inhibition rates ranging from 18.75% to 77.50%, among which IS1, IS2, and IS3 exhibited the highest biocontrol potential.

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