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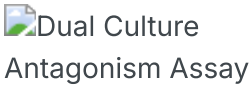
Dual Culture Antagonism Assay



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We use this protocol and it's working

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Keywords: Dual culture assay, antagonism, Pseudomonas auctus JDE115, Agroathelia rolfsii, biocontrol, soil-borne pathogen, plant growth-promoting rhizobacteria (PGPR), fungal suppression, sclerotia, hyphal inhibition, fungal interaction, fungal pathogen, phytopathogenic fungus, borne fungal pathogen, candidatus pseudomonas auctus, jde115 against the phytopathogenic fungus, bacterial biocontrol agent, antagonistic activity of bacterial biocontrol agent, fungus, dual culture antagonism assay, dual culture antagonism assay this protocol, hyphal plugs of the fungus, potato dextrose agar, agroathelia rolfsii, dextrose agar, biocontrol potential

Disclaimer

This protocol is intended for research purposes only. Results may vary depending on laboratory conditions, microbial strains, and experimental design. Users are responsible for ensuring compliance with relevant biosafety and institutional guidelines before implementation.

Abstract

This protocol describes the dual culture antagonism assay used to evaluate the biocontrol potential of *'Candidatus Pseudomonas auctus'* JDE115 against the phytopathogenic fungus *Agroathelia rolfsii* (syn. *Sclerotium rolfsii*). The assay was performed on potato dextrose agar (PDA) under both direct contact and non-contact conditions using sclerotia and hyphal plugs of the fungus. Multiple experimental setups were tested to assess bacterial–fungal interactions, with replicates conducted to ensure reproducibility. Antagonistic effects were evaluated through visual observation, photographic documentation, and radial growth measurements, followed by quantification of growth suppression. This standardized protocol provides a reproducible framework for assessing the antagonistic activity of bacterial biocontrol agents against soil-borne fungal pathogens.

Attachments

DOCX

Dual Culture Antagon...

1.5MB

Guidelines

The antagonistic potential of ‘Candidatus P. auctus’ JDE115 against A. rolfsii was evaluated using a dual culture assay on potato dextrose agar (PDA). PDA was prepared by dissolving 24 g of potato dextrose broth and 15 g of agar in 1 L of deionized water, sterilization at 121°C for 15 minutes. The media was then poured into sterile petri dishes and allowed to solidify.

Seven parameters were tested: (a) JDE115 placed directly on A. rolfsii sclerotia, (b) JDE115 alone, (c) JDE115 and sclerotia placed distantly, (d) sclerotia alone, (e) JDE115 and fungal hyphal plug placed distantly, (f) hyphal plug alone, and (g) JDE115 placed directly on hyphae.

Fresh cultures of JDE115 (OD600 ≈ 0.6) and A. rolfsii (sclerotia or 6–8 mm hyphal plugs) were used in all treatments (Fig. 1). Plates were incubated at 28°C for 7 days. Each treatment included five biological replicates and was repeated independently five times. Fungal growth inhibition was assessed visually and documented photographically to evaluate antagonistic effects under both contact and non-contact conditions (Khan et al. 2018).

In a complimentary dual plate assay, the radial growth of A. rolfsii was measured in centimeters after 7 days of incubation at 28°C. The experiment consisted of two treatments: (1) A. rolfsii control, where sterile water was applied instead of JDE115, (2) JDE115 treatment, in which the bacterium was streaked 4 cm away from the fungal plug (Ali et al. 2020). Each treatment included 10 biological replicates, and the experiment was independently repeated three times under identical conditions.

Fungal inhibition was quantified by calculating the percentage suppression of radial growth using the following formula (Montealegre et al. 2003):

Suppression (%) = (1 – Mean growth in treatment / Mean growth in control) × 100

Materials

- Potato dextrose broth: 24 g per 1 L
- Agar: 15 g per 1 L
- Deionized water: 1 L
- Potato dextrose agar (PDA) plates / sterile petri dishes
- Autoclave (sterilization at 121°C for 15 minutes)
- Fresh culture of JDE115 (OD600 ≈ 0.6)
- A. rolfsii sclerotia or 6–8 mm hyphal plugs
- Sterile water
- Incubator capable of 28°C

Safety warnings

- Handle *Agroathelia rolfsii* (syn. *Sclerotium rolfsii*) with care, as it is a plant pathogen that can spread through contaminated surfaces or soil.
- Dispose of fungal and bacterial cultures according to institutional biosafety guidelines.
- Work under Biosafety Level 2 (BSL-2) practices when handling live cultures.
- Avoid direct contact with fungal structures and bacterial suspensions; wear gloves, a lab coat, and eye protection.

Ethics statement

This study did not involve human participants or vertebrate animals. All experiments were conducted with microbial cultures under the approval and biosafety regulations of Virginia Tech's Institutional Biosafety Committee. No additional ethical approvals were required.

Before start

Ensure all microbial cultures are fresh and prepared under aseptic conditions. Confirm that potato dextrose agar (PDA) and all other media are sterilized and properly solidified before inoculation. Calibrate spectrophotometer for accurate OD600 measurements of bacterial suspensions. Maintain sterile workspace and equipment to prevent contamination that may affect results.

Dual Culture Antagonism Assay

1 Prepare potato dextrose agar (PDA) by dissolving 24 g potato dextrose broth and 15 g agar in 1 L deionized water; sterilize at 121°C for 15 minutes. Pour the sterilized media into sterile petri dishes and allow to solidify.

2 Set up the seven treatment parameters on solidified PDA: (a) JDE115 placed directly on A. rolfsii sclerotia; (b) JDE115 alone; (c) JDE115 and sclerotia placed distantly; (d) sclerotia alone; (e) JDE115 and fungal hyphal plug placed distantly; (f) hyphal plug alone; (g) JDE115 placed directly on hyphae.

3 Use fresh cultures of JDE115 (OD600 ≈ 0.6) and A. rolfsii (sclerotia or 6–8 mm hyphal plugs) for all treatments (Fig. 1).

4 Incubate the inoculated plates at 28°C for 7 days.

5 For the dual culture assay include five biological replicates per treatment and repeat the entire experiment independently five times. Assess fungal growth inhibition visually and document photographically to evaluate antagonistic effects under both contact and non-contact conditions.

6 Perform the complementary dual plate (radial growth) assay: after 7 days incubation at 28°C measure radial growth of A. rolfsii in centimeters. Use two treatments: (1) A. rolfsii control — apply sterile water instead of JDE115; (2) JDE115 treatment — streak JDE115 4 cm away from the fungal plug. Use 10 biological replicates per treatment and repeat the experiment independently three times under identical conditions.

7 Quantify fungal inhibition by calculating percentage suppression of radial growth using the formula: Suppression (%) = (1 − Mean growth in treatment / Mean growth in control) × 100.

Protocol references

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