



Phenotypic Variation and ROS Tolerance in *Paenibacillus* Isolates with Contrasting Host Outcomes



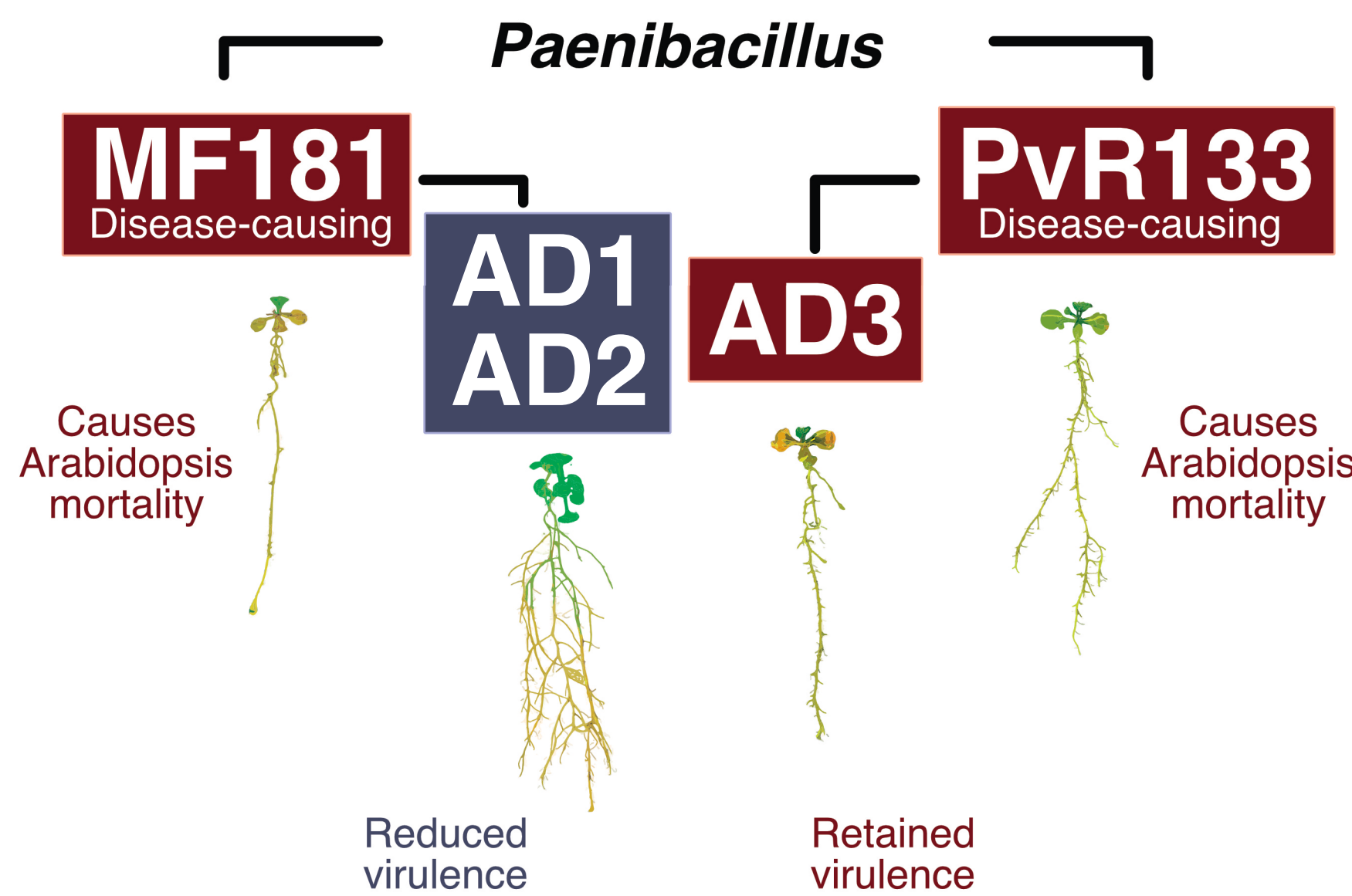
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Introduction

Plant-associated microbiomes play important roles in plant health and resilience. *Paenibacillus* includes both plant growth-promoting and pathogenic strains, but the factors underlying these contrasting host outcomes remain poorly understood. We investigated whether colony morphological variation contributes to differences in ROS tolerance and plant phenotypes.



Do changes in colony morphology affect the pathogenicity of *Paenibacillus*?

Colony morphological variation drives phenotypic change *in planta*

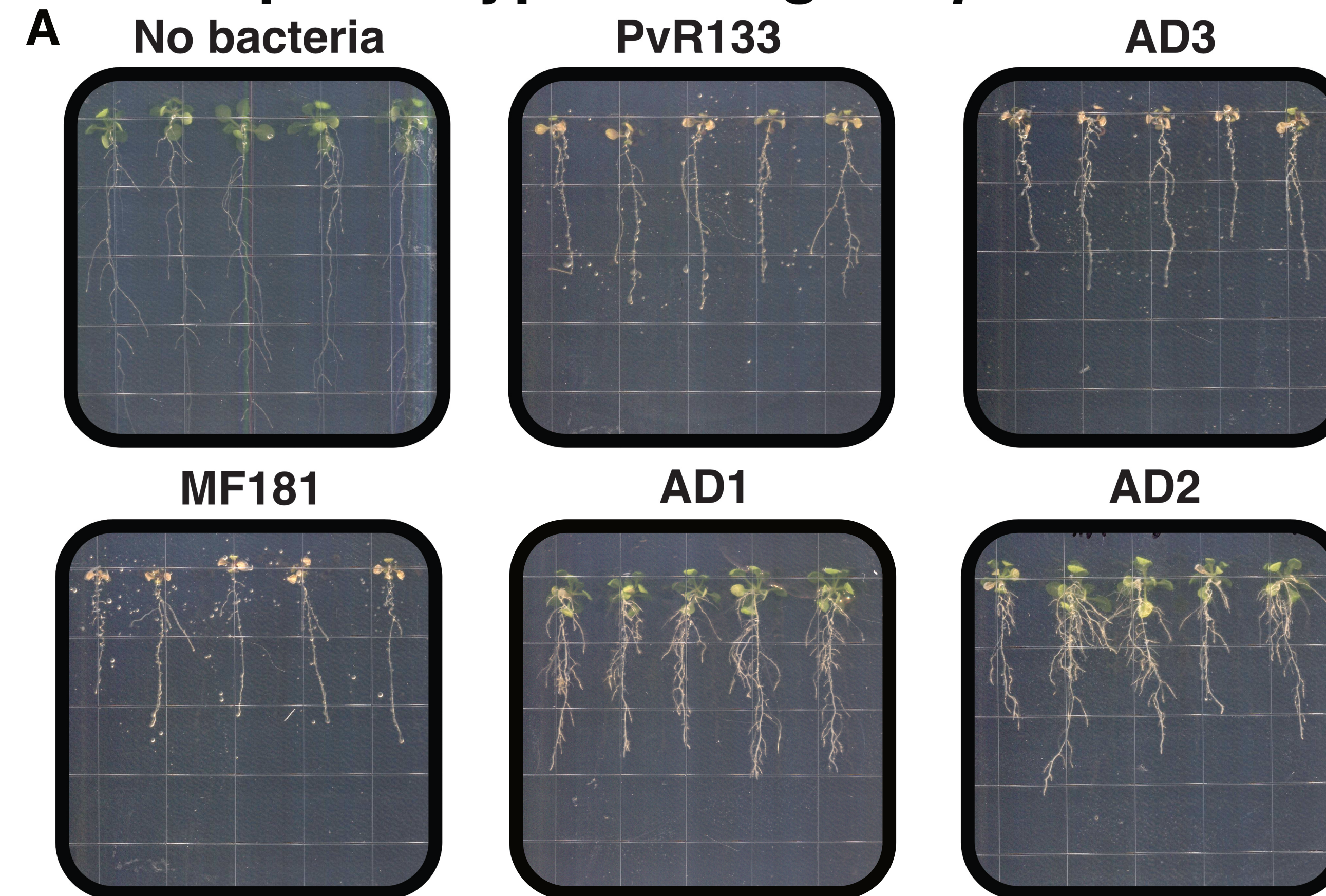


Fig 1. (A) *Arabidopsis thaliana* seedlings 12 days post-inoculation with wild-type *Paenibacillus* isolates (MF181, PvR133) or their morphology variants (AD1, AD2, AD3), compared to an uninoculated control.

Changes in morphology do not impact growth *in vitro*

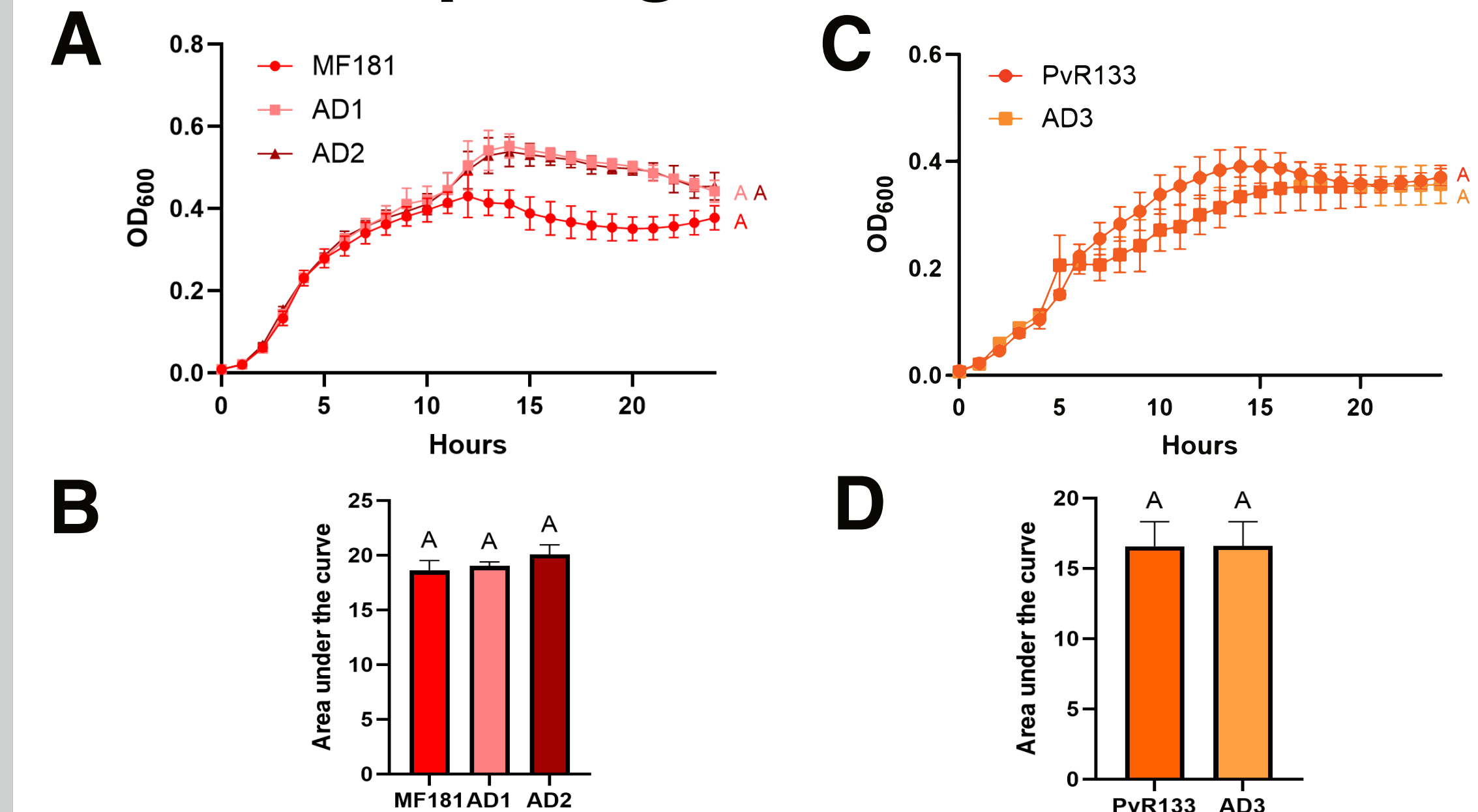
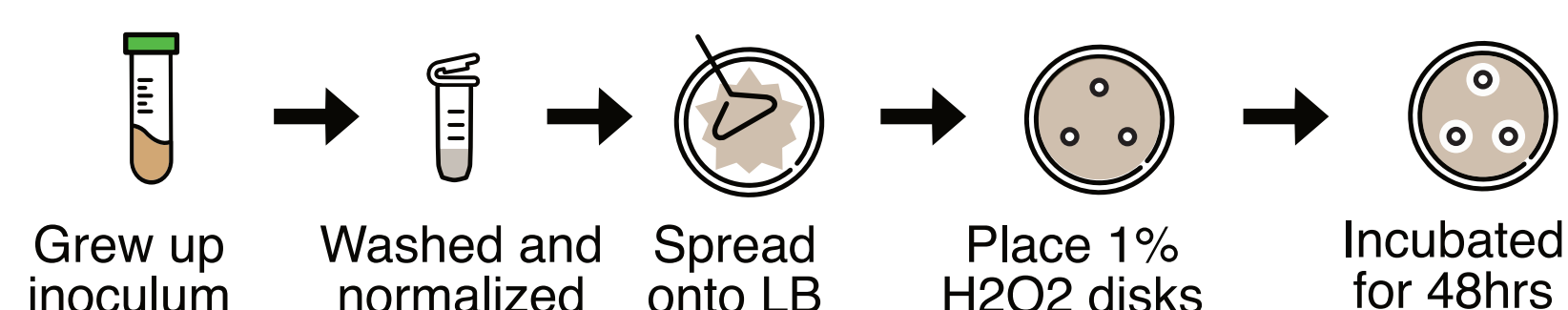


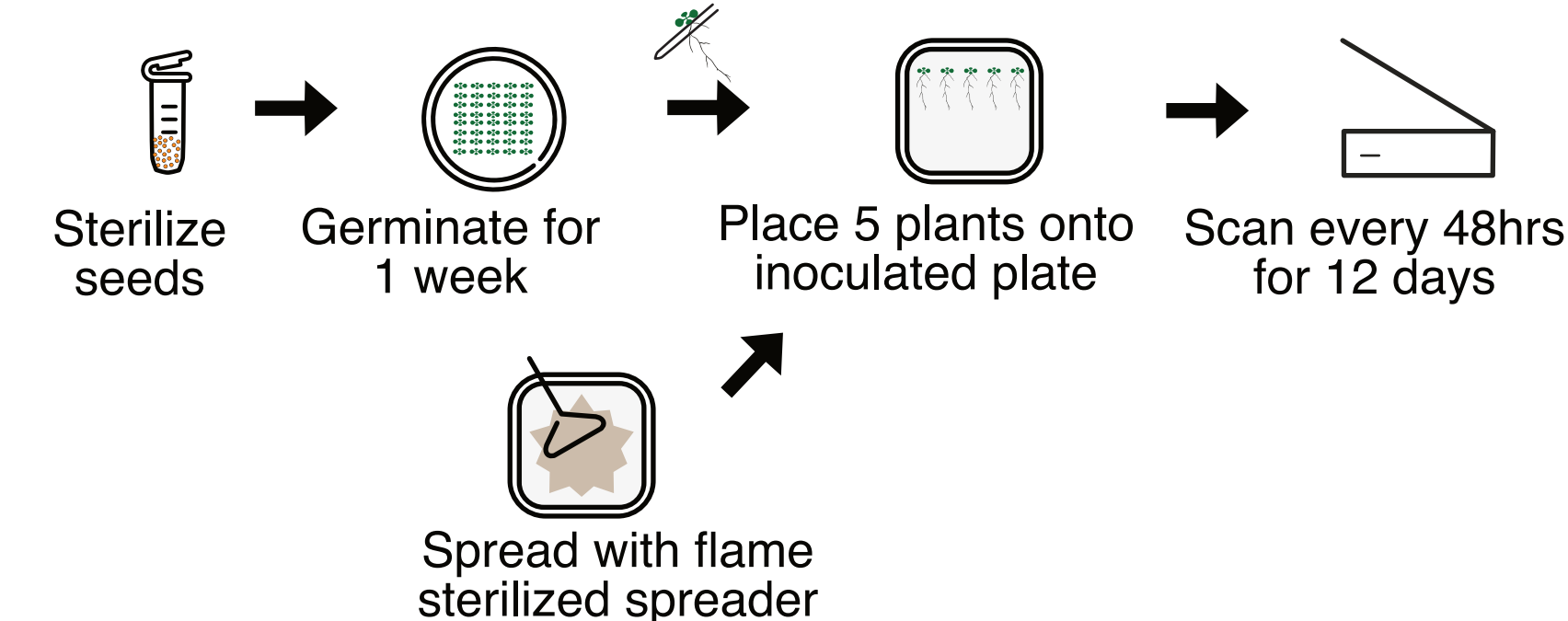
Fig. 3. (A, C) Growth curves (OD₆₀₀ vs. time, h) of MF181 and its variants (AD1, AD2) and of PvR133 and its variant (AD3) in lysogeny broth. Curves represent the mean of three biological replicates. Statistical comparisons of growth were performed using the terminal (final) time point, with significance among the MF181 variants assessed using a Kruskal–Wallis test followed by Dunn's multiple-comparisons test and differences between PvR133 and AD3 assessed using a Mann–Whitney U test. **(B, D)** Area under the growth curve (AUC) corresponding to panels A and C. Statistical significance for the AUC analyses was assessed using the same tests.

Methods

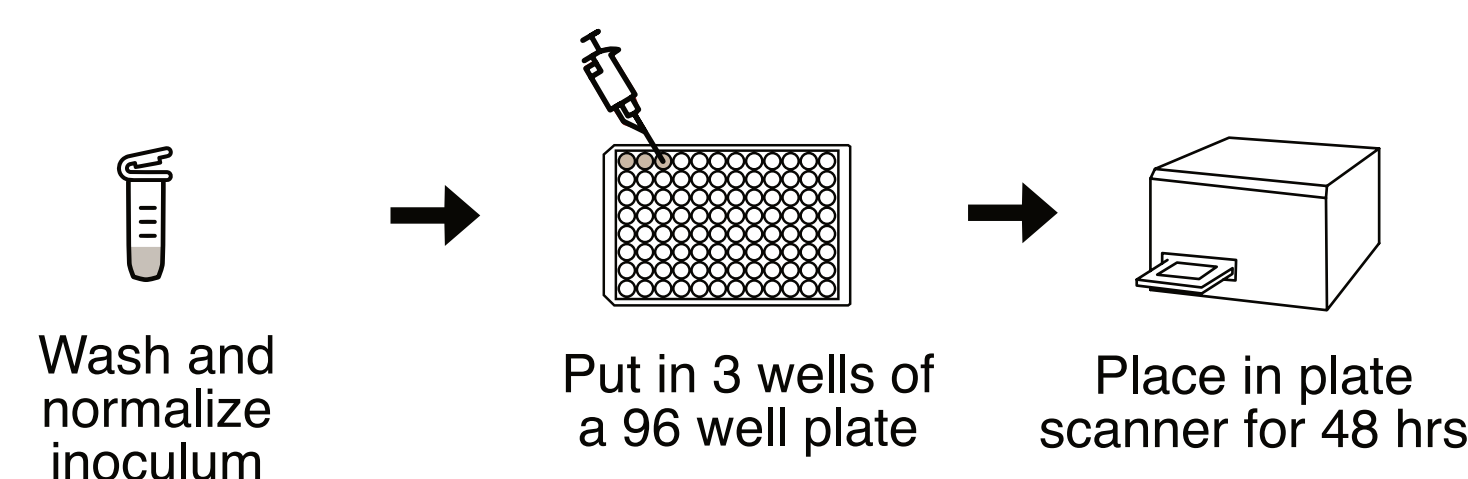
ROS



VERTICAL PLATE



Growth Curve



ROS resistance is unlikely to be the mechanism causing alteration of pathogenicity

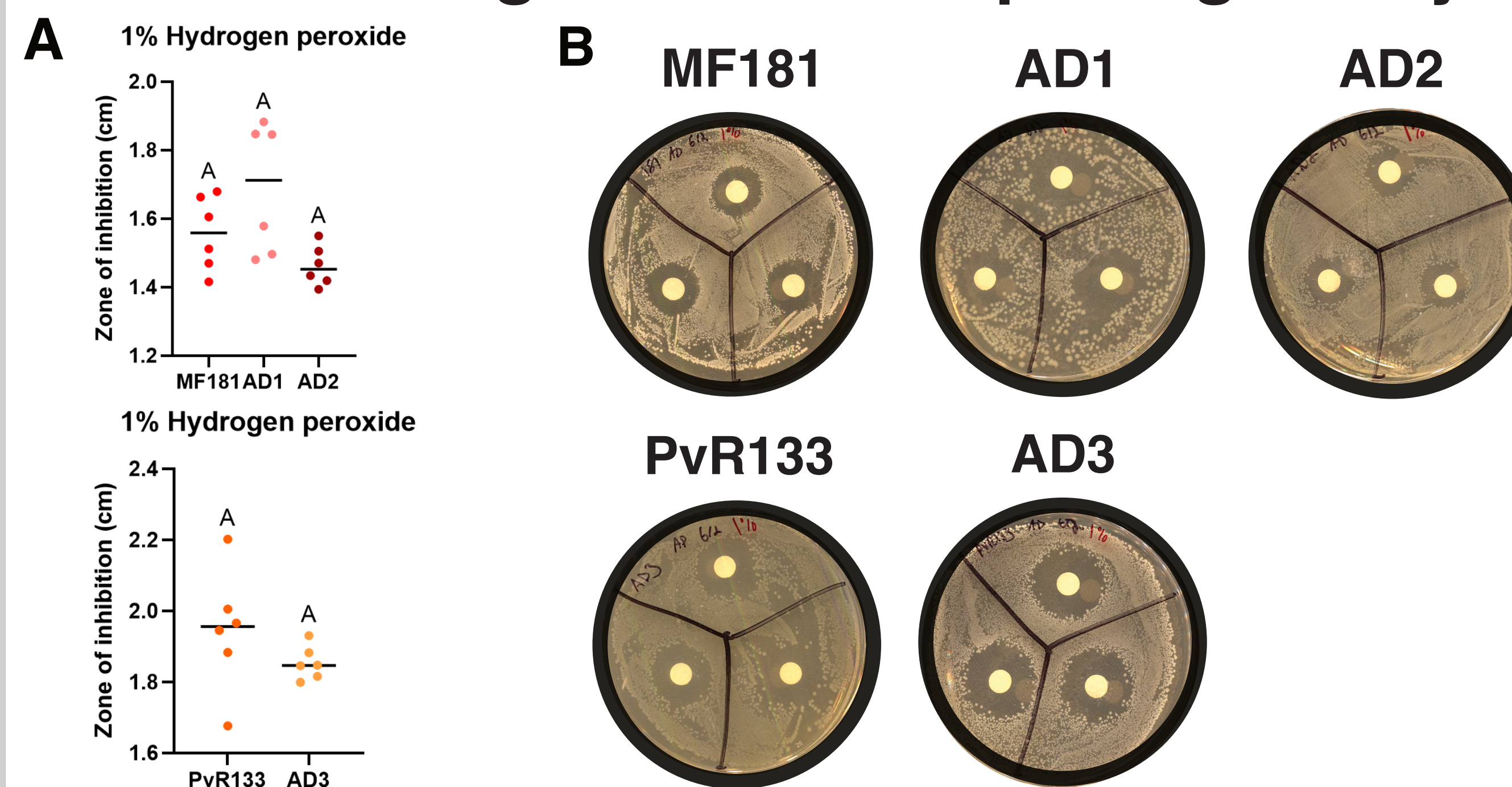


Fig. 2. (A) Diameter of zones of inhibition (cm) following application of 1% H₂O₂ disks to plates inoculated with MF181, AD1, AD2, PvR133, and AD3. Statistical significance for the MF181 variants was assessed using a Kruskal–Wallis test followed by Dunn's multiple-comparisons test. Differences between PvR133 and AD3 were assessed using a Mann–Whitney U test. **(B)** Representative scans of inhibition zones after 48 h.

Future Directions

- RNA sequencing on MF181, PVR133, and variants
- Test on other *Arabidopsis* phenotypes
- Biolog plates to assess metabolic differences
- *In planta* assays in other species, e.g. switchgrass, pictured below

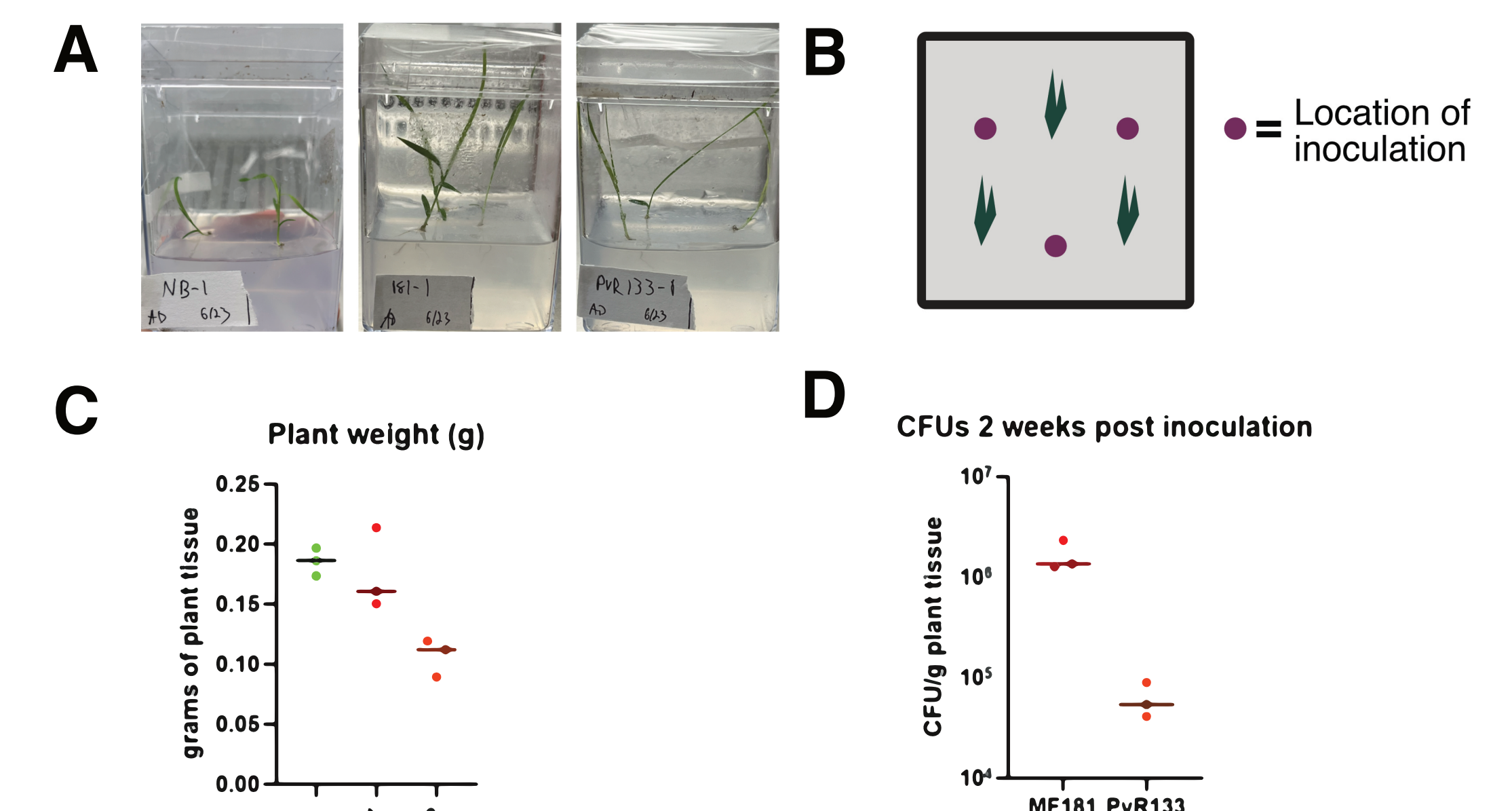


Fig 4. (A) Containers with switchgrass with no added bacteria, MF181, or PvR133. **(B)** Inoculation and growth setup in containers from (A). **(C)** Plant weight after 2 weeks. **(D)** CFUs derived from homogenized and diluted inoculated plant material.

Acknowledgements

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Citations

Lee, Y., Balaraju, K., Kim, S.-Y., & Jeon, Y. (2022). Occurrence of phenotypic variation in *Paenibacillus polymyxa* E681 associated with sporulation and carbohydrate metabolism. *Biotechnology Reports*, 34, e00719. <https://doi.org/10.1016/j.btre.2022.e00719>