

Introduction

- Microbes live in a plant's seeds, with impacts on seed viability [1,2].
- Seed microbes can be acquired from the parent stigma by exposure to the wind, contact with pollinators, and other processes [3].
- Studies on how microbe-microbe interactions impact microbial fitness and transmission to seeds are limited [4,5].
- **Research question:** Are there antagonistic and/or facilitative interactions between microbes found in seeds and floral stigmas of watermelon (*Citrullus lanatus*)?

Methods

- We tested for interactions between microbial strains isolated from watermelon seeds, fruits and stigmas (Table 1). *Pantoea agglomerans* was included as an outgroup microbe.

Table 1. The different microbial used in this experiment, including their type (kingdom and growth form), where they were isolated and the study where they were first reported.

Microbe (isolate ID)	Microbe type	Isolation source	Ref
<i>Bacillus</i> (T.m10.b1)	Bacterium	Watermelon seed	[2]
<i>Cladosporium</i> (T.m8.f1)	Mold fungus	Watermelon seed	[2]
<i>Acidovorax citrulli</i> (AAC00-1R)	Bacterium	Watermelon fruit	[1]
<i>Erwinia</i> (P.s8.b2)	Bacterium	Watermelon stigma	[2]
<i>Fusarium</i> (P.s6.f4)	Mold fungus	Watermelon stigma	[2]
<i>Paraburkholderia</i> (T.s1.b2)	Bacterium	Watermelon stigma	[2]
<i>Rosenbergiella</i> (P.s10.b1)	Bacterium	Watermelon stigma	[2]
<i>Starmerella</i> (T.s10.f3)	Yeast fungus	Watermelon stigma	[2]
<i>Pantoea agglomerans</i> (Pa299R)	Bacterium	Pear leaf	[3]

- We plated microbes on media that simulate the sugars available on watermelon stigmas (WSSM).
- We plated microbes in three different configurations based on the types of microbes being paired (Figure 1).

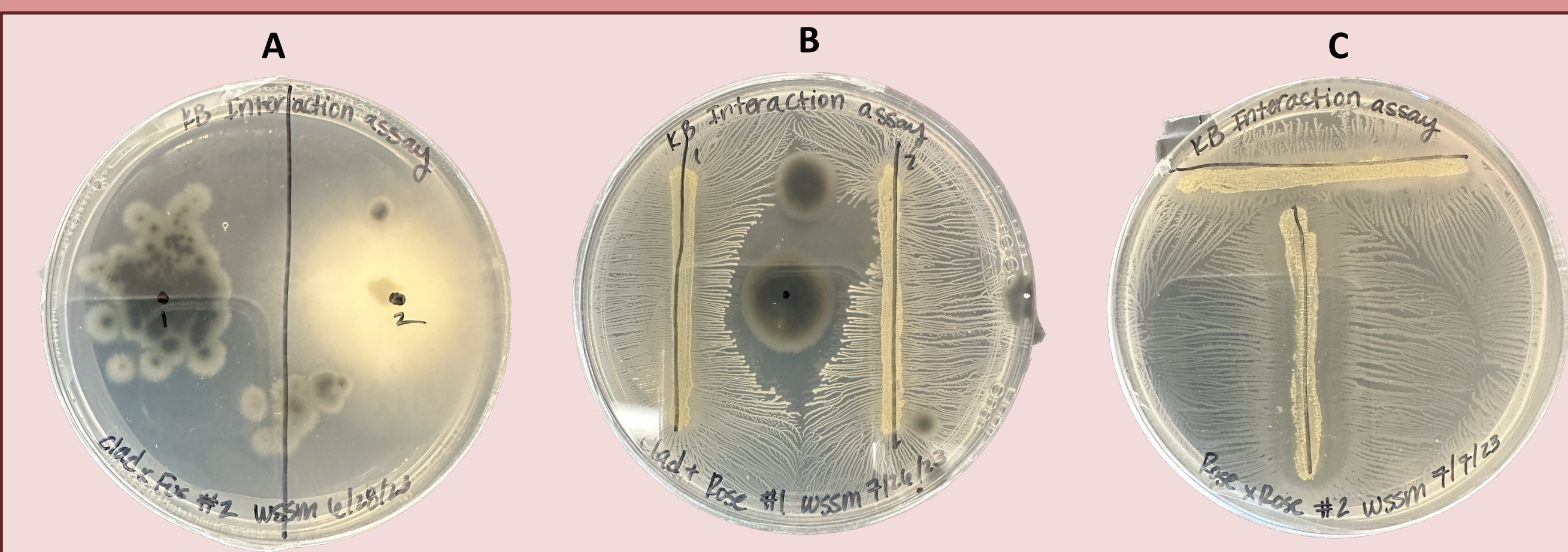


Figure 1. Culturing configurations for pairing mold with mold (A), bacteria or yeast with mold (B) and bacteria/yeast with bacteria/yeast (C).

- Plates were left to incubate on ambient light and temperature for 7 days.
- On day 7, we recorded the growth of each microbe at its widest point (i.e. maximum growth) and gave it an interaction score (+1 = growing towards the other microbe, 0 = growing neither towards or away from the other microbe, -1 = growing away from the other microbe).

Results

There were significant differences in maximum growth for each isolate across pairwise combinations (Table 2). These differences included both increased and decreased maximum growth compared to controls (Figure 2).

Table 2. Results of Kruskal-Wallis rank sum tests on the variation in maximum growth for each focal microbe. χ^2 refers to the chi-squared test statistic, df refers to the degrees of freedom, and the p-value refers to the significance of the χ^2 value. Significant p-values (i.e. below 0.05) are shown in bold.

Microbe (isolate ID)	χ^2	Df	p-value
<i>Bacillus</i> (T.m10.b1)	21.167	8	0.006716
<i>Cladosporium</i> (T.m8.f1)	17.998	8	0.02124
<i>Acidovorax citrulli</i> (AAC00-1R)	20.732	8	0.007893
<i>Erwinia</i> (P.s8.b2)	23.154	8	0.003172
<i>Fusarium</i> (P.s6.f4)	19.467	8	0.01255
<i>Paraburkholderia</i> (T.s1.b2)	28.239	8	0.0004309
<i>Rosenbergiella</i> (P.s10.b1)	16.849	8	0.03173
<i>Starmerella</i> (T.s10.f3)	28.212	8	0.0004356
<i>Pantoea agglomerans</i> (299R)	21.78	8	0.005341

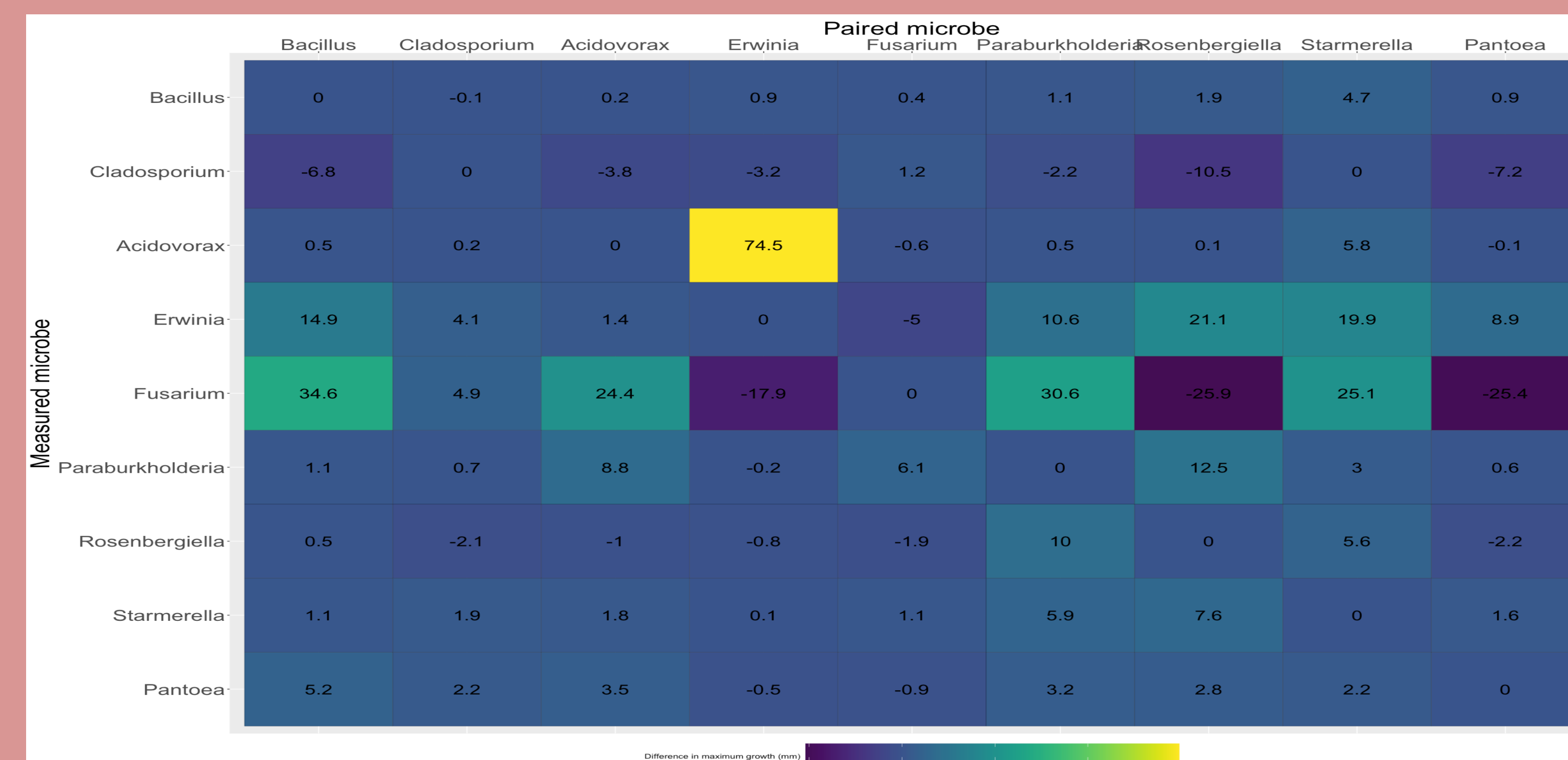


Figure 2. A heatmap of the difference in average maximum growth between controls and each microbial pairing. The number in each cell is the calculated difference in average maximum growth between the pairing and the control in millimeters.

Positive and negative interactions were observed between various microbe pairings (Figure 3). We observed trends of negative interactions between seed-borne isolates and stigma-borne isolates.

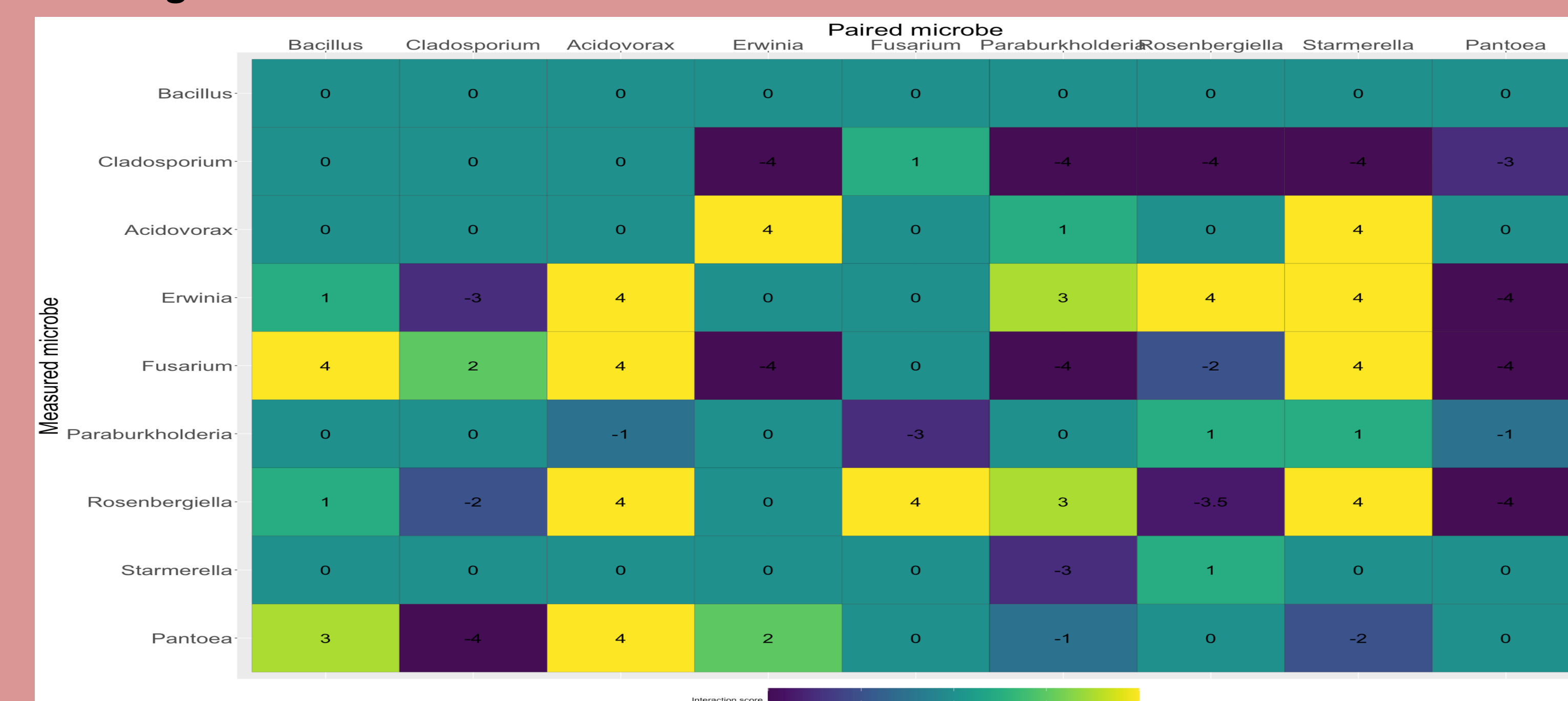


Figure 3. A heatmap of the cumulative interaction scores for each focal microbe in all pairing combinations. The number in each cell is the cumulative interaction score for that pairing.

Conclusions

- *Bacillus* is an antagonistic microbe and is used as a biopesticide for *Fusarium* [9]. However, in our experiment, the *Fusarium* was able to grow on and around the *Bacillus*. This could be because of the method, source culture age, or cell concentration that we used.
- *Erwinia* has previously been studied for biocontrol and was one of our microbes that had the most growth when paired with *Acidovorax* and caused the *Fusarium* to have an avoidant reaction [9].
- *Pantoea agglomerans*, another bacterium used for biocontrol [10], had antagonistic interactions with *Fusarium* and *Erwinia*.

Future directions

- Test if microbes antagonize each other via direct contact or through secreting antibiotic chemicals into the media and air
- Test for interactions quantitatively by measuring the area of inhibition zones between microbe pairs
- Test if these interactions affect transmission from stigmas to seeds
- Test if antagonistic microbes can reduce effects of putative pathogens on seed viability

References

1. Chesneau, G., Laroche, B., Prévieux, A., Marais, C., Briand, M., Marolleau, B., Simonin, M., & Barret, M. (2022). Single Seed Microbiota: Assembly and Transmission from Parent Plant to Seedling. *Mbio*, 13(6), e01648-22.
2. Pal, G., Kumar, K., Verma, A., & Verma, S. K. (2022). Seed inhabiting bacterial endophytes of maize promote seedling establishment and provide protection against fungal disease. *Microbiological Research*, 255, 126926.
3. Shade, A., Jacques, M.-A., & Barret, M. (2017). Ecological patterns of seed microbiome diversity, transmission, and assembly. *Current Opinion in Microbiology*, 37, 15-22.
4. Raghavendra, A. K. H., Newcombe, G., Shipunov, A., Baynes, M., & Tank, D. (2013). Exclusionary interactions among diverse fungi infecting developing seeds of *Centaurea stoebe*. *FEMS Microbiology Ecology*, 84(1), 143-153.
5. Newcombe, G., Marlin, M., Barge, E., Heitmann, S., Ridout, M., & Busby, P. E. (2022). Plant Seeds Commonly Host *Bacillus* spp., Potential Antagonists of Phytopathogens. *Microbial Ecology*.
6. Walcott, R. R., Fessehale, A., & Castro, A. C. (2004). Differences in Pathogenicity between two Genetically Distinct Groups of *Acidovorax* avenae subsp. *Citrulli* on Cucurbit Hosts. *Journal of Phytopathology*, 152(5), 277-285.
7. Lui, K., AM Velasco, GE Bergmann, R Mahejan, RL Vannette and JHJ Leveau. 2023. What microbes are found in watermelon (*Citrullus lanatus*) flowers and seeds? URSCA Conference, Davis, CA.
8. Remus-Emsermann MNP, Kim EB, Marco ML, Tecón R, Leveau JHJ. 2013. Draft genome sequence of the phyllosphere model bacterium *Pantoea agglomerans* 299R. *Genome Announc.* 1(1):e00036-13
9. Khalaf, E. M., & Raizada, M. N. 2018. Bacterial seed endophytes of domesticated cucurbits antagonize fungal and oomycete pathogens including powdery mildew. *Frontiers in Microbiology*, 9.
10. Pusey, P. L., Stockwell, V. O., Reardon, C. L., Smits, T. H. M., & Duffy, B. (2011). Antibiosis Activity of *Pantoea agglomerans* Biocontrol Strain E325 Against *Erwinia amylovora* on Apple Flower Stigmas. *Phytopathology*, 101(10), 1234-1241.

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