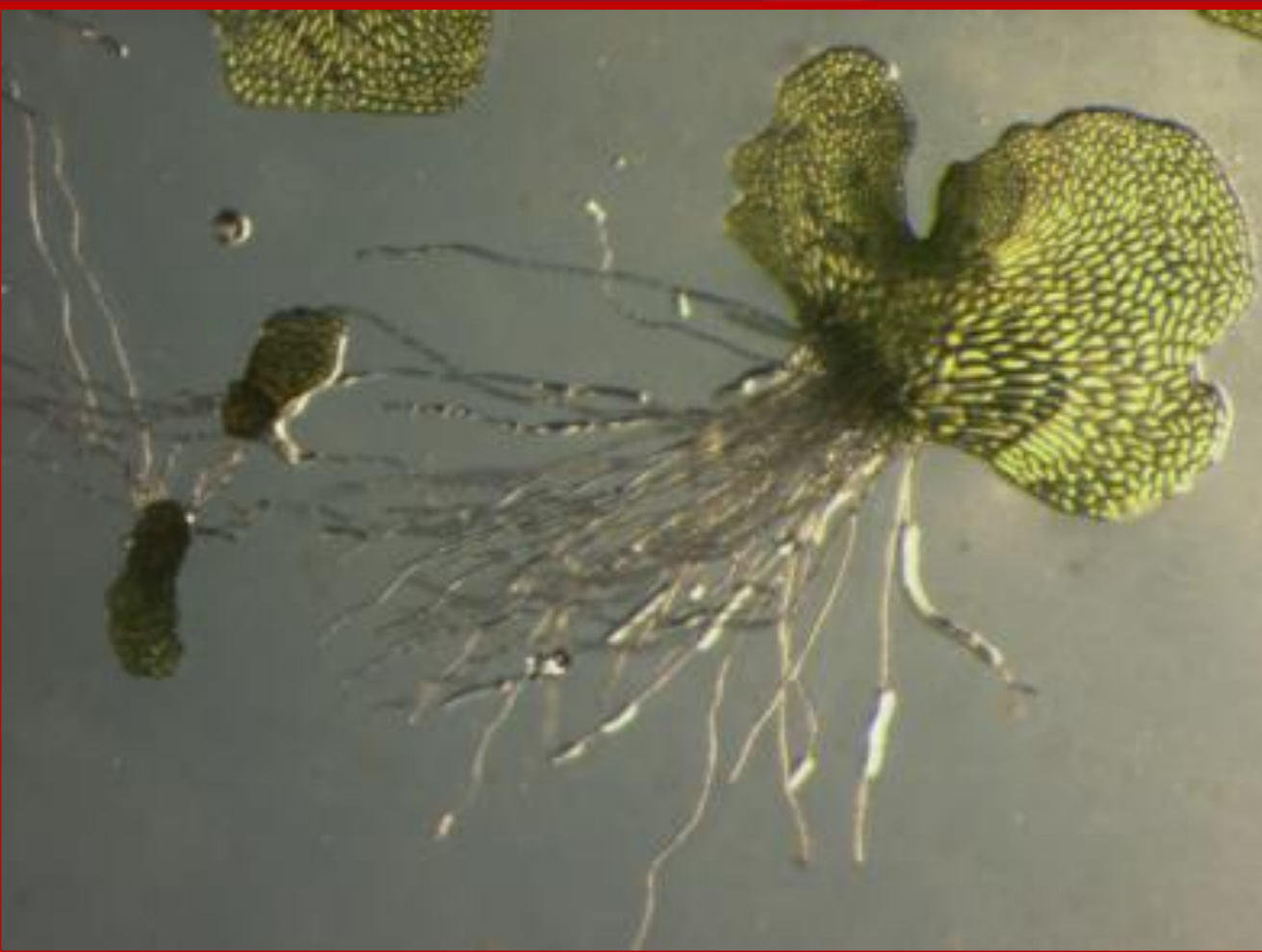


A diverse group of soil bacteria alter rhizoid development in gametophytes of the fern *Ceratopteris richardii*

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Introduction:

Vascular seedless plants such as ferns undergo an alternation of generations during their life cycle. Although most of their life cycle is spent in the diploid phase, they also grow and reproduce in the haploid phase. Diploid sporophyte plants produce and release haploid spore cells by means of meiosis. These haploid cells can then undergo mitosis to produce a smaller multicellular gametophyte that is able to undergo regular metabolism and photosynthesis (Spiro 2008). In fern species *Ceratopteris richardii* (C-fern), gametophytes can mature into either males or hermaphrodites. Male gametophytes mature in the presence of hormone antheridiogen, which is excreted by mature hermaphroditic gametophytes. Gametophytes that are not exposed to the hormone mature into hermaphrodites (Ganger 2019 , Figure I).

Many plants, including C-fern, require mutualistic interactions and relationships with soil bacteria in order to successfully grow and reproduce either directly or indirectly attached to the plant itself (Heiden 2016). Soil bacteria either work to supply nutrients to plants, stimulate plant growth and aid in prevention of pathogens into plants. Additionally, soil bacteria can directly interact with soil bacteria through secretion of plant hormones (Goh 2013). Previous studies (Ganger 2019) showed exposure of C-fern to an isolated strain of soil bacteria *Pseudomonas nitroreducens* resulted in dramatic increase of rhizoid length as well as number of hermaphrodites in cultures. Here we explore the extent in which various strains of bacteria influence gametophyte development, including rhizoid length to further develop potential mechanisms and pathways in which the two organisms can interact.

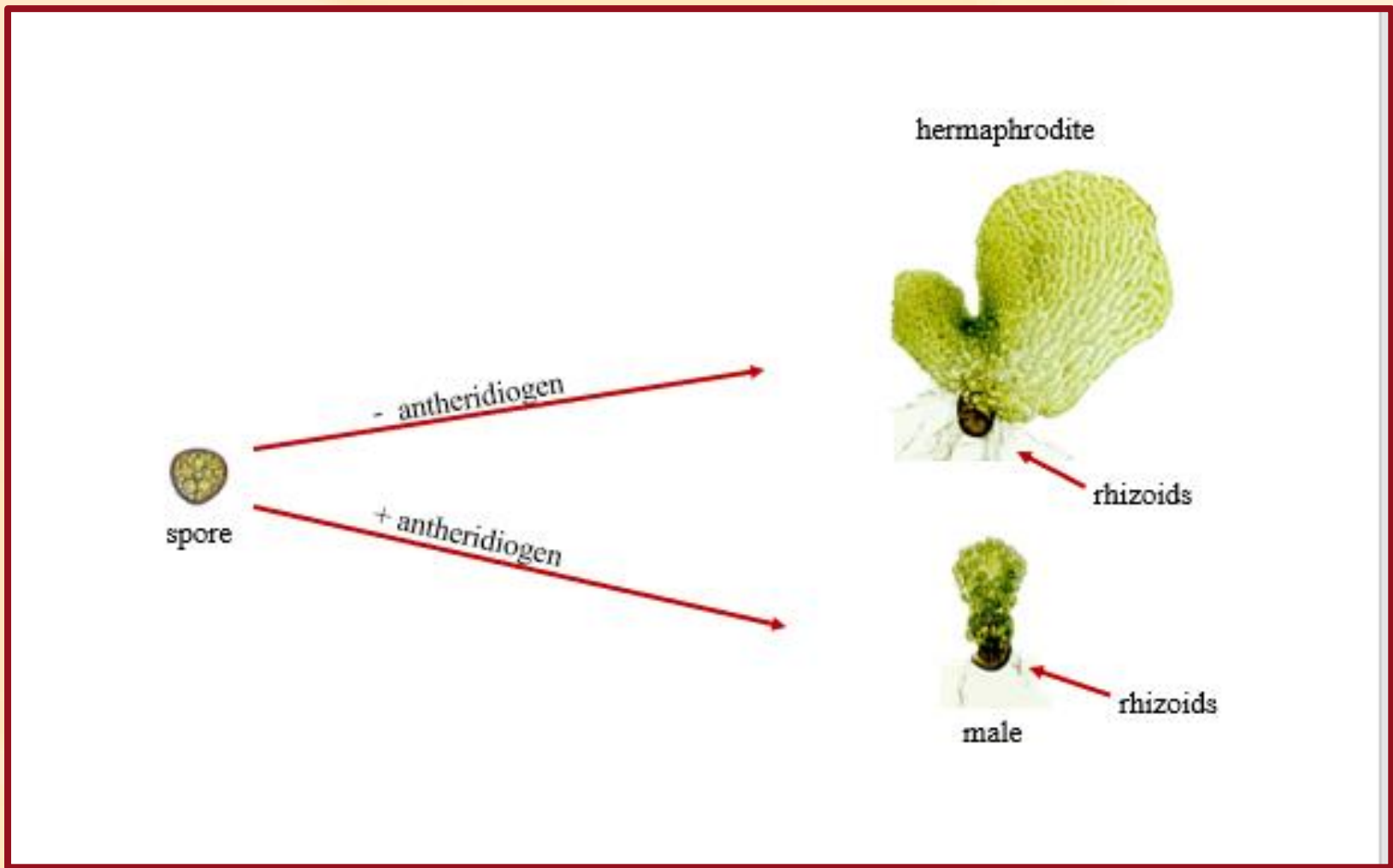


Figure I: Sex Differentiation in C-Fern Gametophytes

Bacterial Effects on Rhizoid Length

How do various bacteria affect gametophyte development?

Materials and Methods:

Fifteen soil bacteria were isolated from local fern roots and three lab strains were introduced to agar plates following protocol from Ganger et al. (2019). Bacteria were grown for 72 hours in tryptic soy broth and then 40 μ l of bacteria were introduced to 40 mL of cooling agar. Multiple different species of both gram-negative and gram-positive soil bacteria were isolated and grown with C-fern spores (Table I) along with three lab strains of bacteria to identify if differences in bacterial structure has an effect on rhizoid length and overall gametophyte development. Petri plates with only tryptic soy broth (TSB) and C-fern spores acted as a control to compare the effects that different bacteria have on rhizoid length. Treatments were triplicated to ensure accuracy of results. C-Fern were allowed to grow until developed thallus shape and external rhizoids (approximately 8 days), and the longest rhizoid as well as area of thallus were measured over the course of 10 days.

The observed thallus area and rhizoid lengths were plotted (Figure II) Because larger gametophytes tend to have larger thallus and rhizoid lengths compared to smaller gametophytes, the log of thallus area was used as a covariance to attribute this natural variance in size of rhizoid and size of gametophyte (Figure III).

Gram Stain	Species/Code	Max. Rhizoid length (mm)
G+	Osen 2-7	0.626
G+	Oreg 4	0.753
G+	Di6	0.509
G+	Lysinibacillus xylanilyticus	01.076
	TSB/Control	0.745
G+	Bacillus aryabhattai	1.397
G-	Osen 1-9	2.336
G-	Pseudomonas plecoglossicida	3.323
G-	Lysinibacillus fusiformis	0.637
G-	Aeromonas eucenrophila	2.870
G-	Bacillus megaterium	1.559
G-	Pantoea vagans	3.355
G-	Pseudomonas nitroreducens	3.455
G-	Dry5	3.498
G-	Osen 2-8	3.001
G-	Pantoea agglomerans	3.605

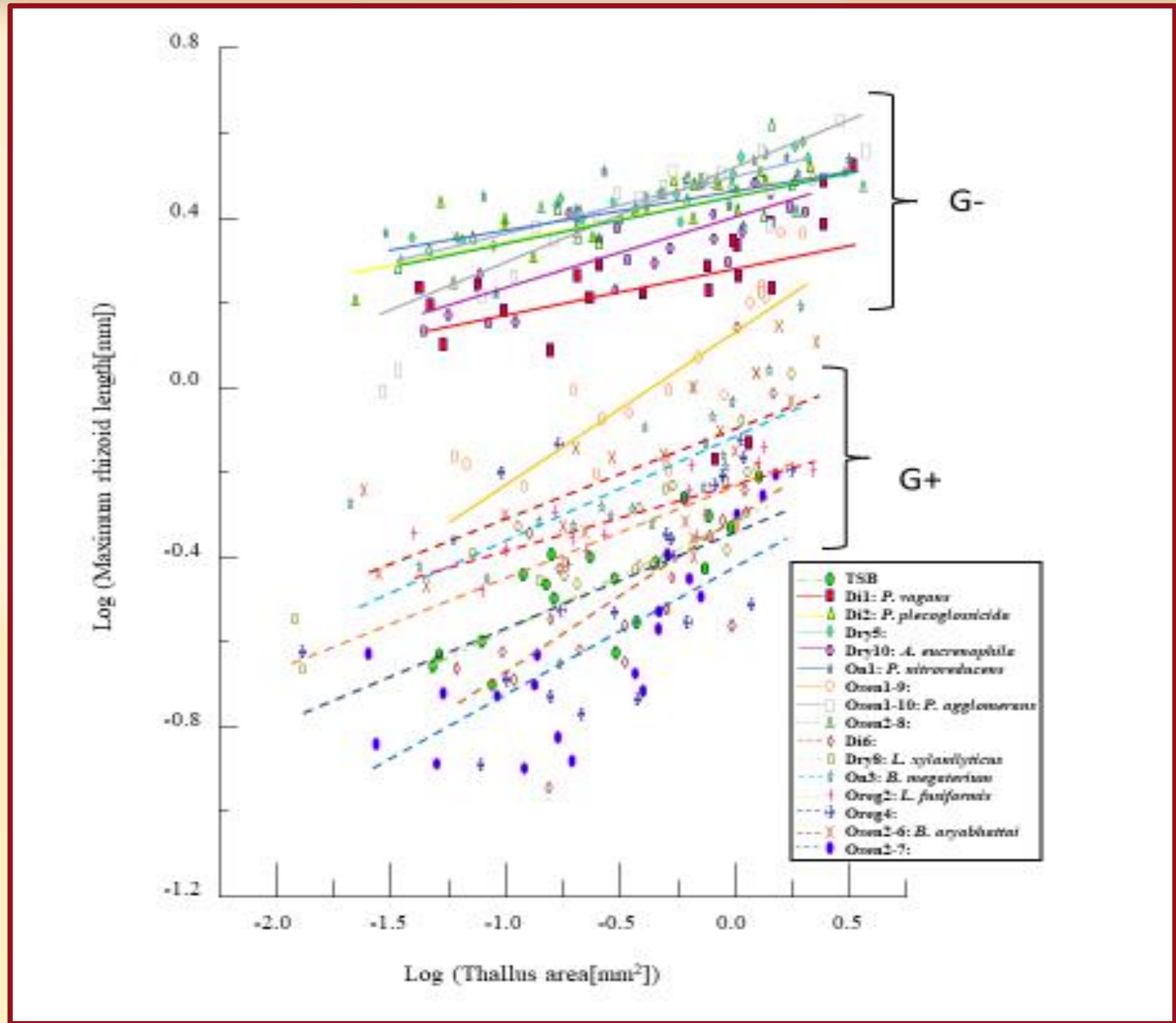


Figure II: Graphical Analysis of the thallus area and rhizoid length for each bacteria treatment.

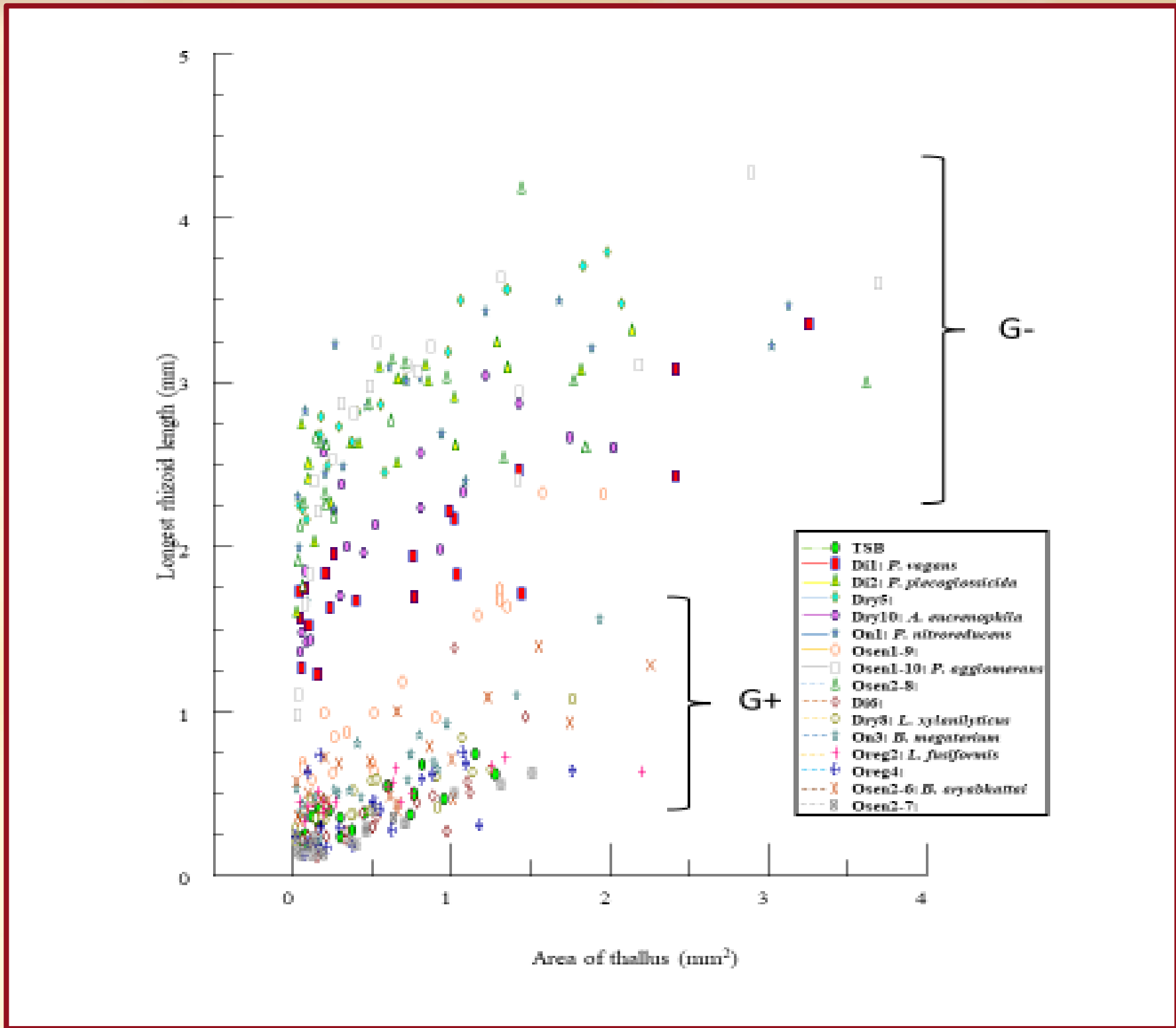


Figure III: Graphical Analysis of the log(thallus area) and log(rhizoid length) for each bacteria treatment.

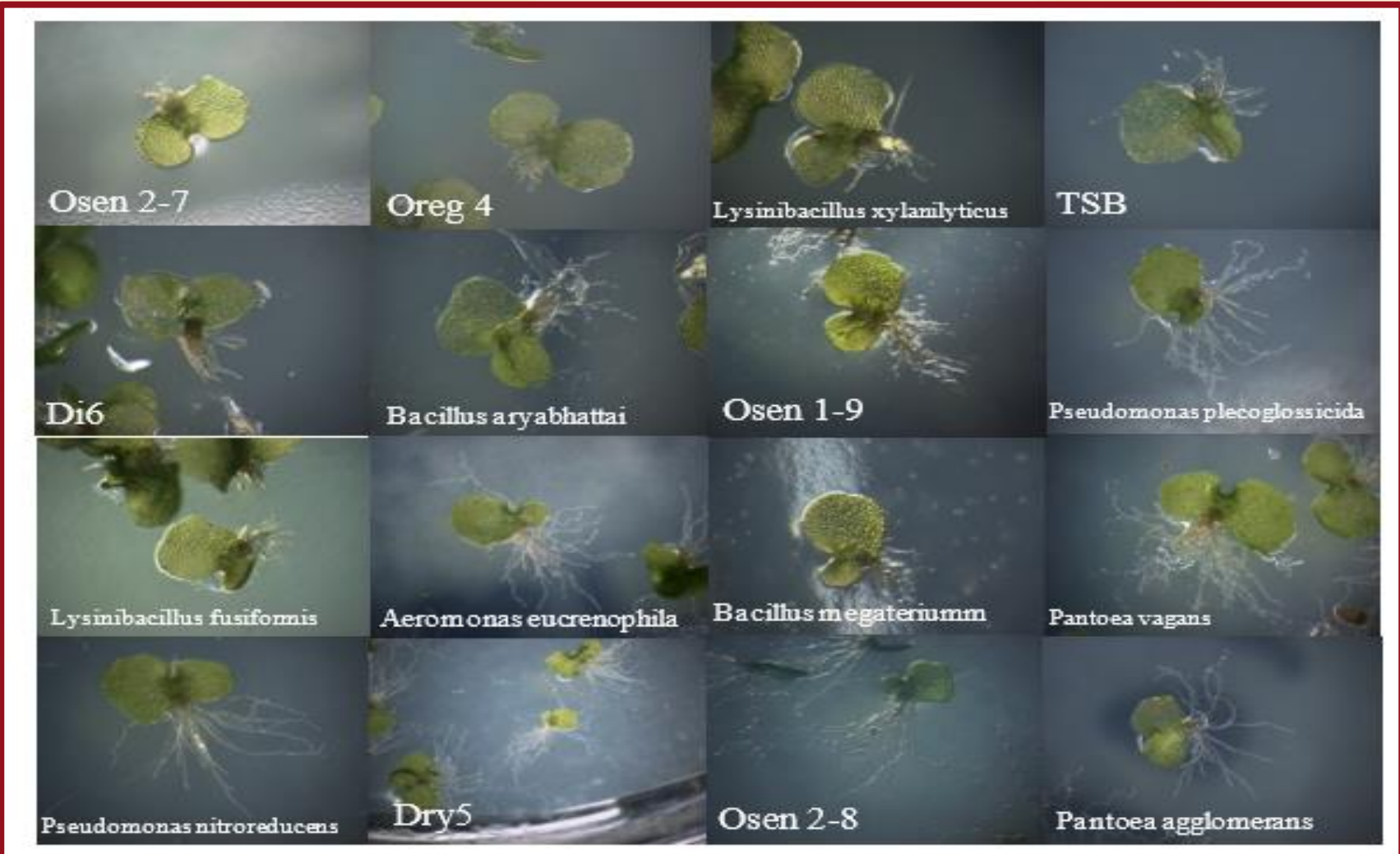


Figure IV: Effects of Gram-negative and Gram-positive Soil Bacteria Strains on Gametophyte and Rhizoid Development

Results and Discussion:

Overall, gram-negative bacteria tended to induce longer rhizoids than gram-positive bacteria. All of the gram-negative bacteria increased the length of rhizoids beyond the axenic control, while many gram-positives did not, although there were notable exceptions. Two gram-positive species, *Bacillus aryabhattai* and *Bacillus megaterium*, increased rhizoid length, while an unidentified gram-positive decreased rhizoid length compared to controls. Additionally, there were differences in the strength of the effect among both gram-positive and among gram-negative species. Some species were more capable of altering rhizoid development than others. This suggests that perhaps the C-fern are sensing the presence of the gram-negative bacteria using a property unique to the microbes. This may also suggest that some species of bacteria have a greater affinity to C-fern and causes a greater effect on gametophyte development, in turn.

One possibility is that the presence of lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria might act as a signal that induces longer rhizoids in C-fern. Variation in effect of gram-negative bacteria may also be contributed to an outer membrane protein that is evolutionarily conserved in all gram-negative microbes. Alternatively, the plants could be sensing the presence of the bacteria using flagellar proteins or the bacteria may be either producing or degrading plant hormones that alter rhizoid development.

Future Experiments

How do we know what is causing developmental changes?

Previous experiments (Chu et al. 2020) have shown that the release of volatile organic compounds (VOC) such as 2,3-butanediol, 3-hydroxy-2-Butanone and 2-pentylfuran produced by *Pseudomonas* spp. resulted in significant plant growth of *Arabidopsis*, including root length development. Because of the similarity in root and rhizoid structure as well as function, excreted VOCs by different bacteria species may induce similar results. A future experiment is currently in progress that tests this hypothesis. Petri dishes are treated with either nutrient-rich TSA agar or nutrient-poor C-fern agar following Ganger et al. (2019). Dishes are then treated with either bacteria of various different concentrations or C-fern spores. These petri dishes are placed open in larger petri dishes before being closed and incubated (Figure V). Petri dishes with no bacteria present acted as controls to observe the effect of gaseous molecules on rhizoid development.

Additionally, other studies indicate that auxin-producing bacteria have an effect on root length and development of plants (Remans et al.). Therefore, bacteria- producing auxin may be inducing greater rhizoid length in C-fern to exchange important biological molecules for overall development and maintenance of bacteria cells. In the future, exposing C-fern to auxin may be considered as a possible explanation for observed rhizoid development. Testing and learning more of the physiological processes of soil bacteria used in lab may reveal a common trait or ability in the bacteria that induce longer rhizoids in C-fern.

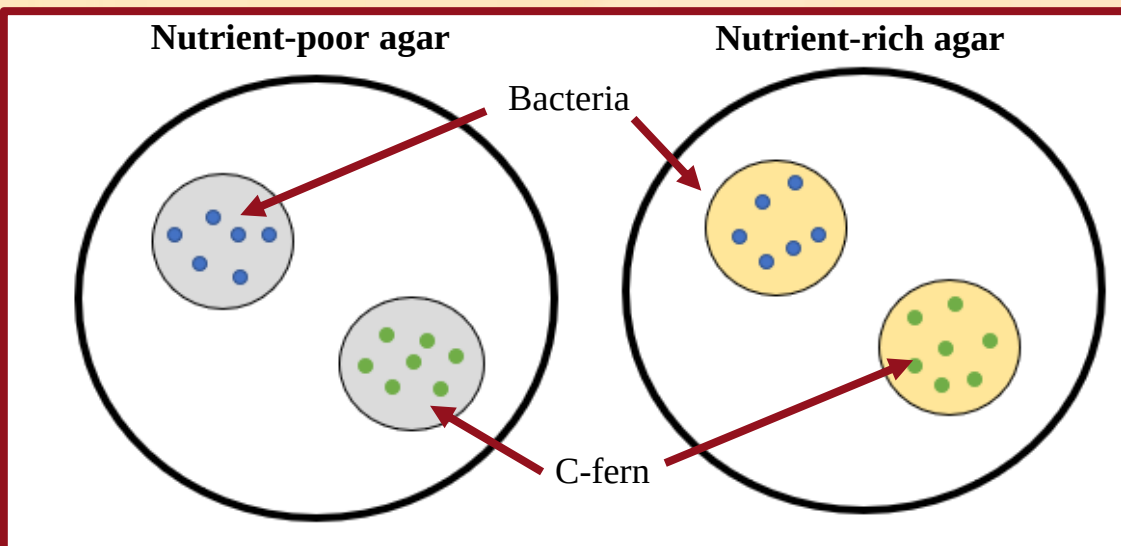


Figure V: Graphical Representation of the experimental setup used to test the effect of potential VOCs on gametophyte development.

Acknowledgements

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