Microbial Worlds Within: SEM of *Darlingtonia* Pitchers

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Abstract

Darlingtonia californica relies on microbes within its pitcher fluid to break down captured prey into usable nutrients. This process is typically attributed to bacteria; however, fungi, are also found in the fluid, and their role remains largely unknown.
For this project, scanning electron microscopy (SEM) was used to directly visualize microorganisms in pitcher fluid and on pitcher tissue. Cultured bacterial and fungal isolates from *Darlingtonia's* fluid provided morphological references for identification. Tissues from the hood and lower pitcher were then processed and examined for comparison.

Both bacteria and yeast-like fungi were observed in each region. The abundance of fungi found means that they are likely integral to the digestive system. These results indicate that yeasts may be overlooked contributors to prey decomposition and nutrient cycling, pointing to a more complex digestive network within *Darlingtonia* that warrants further study.

Introduction

Figure 1: Picture of Darlingtonia californica with arrows pointing to structures that were examined.
Image courtesy of myhomenature.com

Darlingtonia californica is a carnivorous pitcher plant found in northern California and southern Oregon. It captures insects in a fluid-filled trap and relies on microbial residents within its pitcher fluid to convert prey into nutrients[1]. Decomposition in pitcher systems is commonly attributed to bacteria; however, fungal taxa are frequently reported within the pitcher fluid[2]. Despite fungi's well-known role as decomposers in other ecosystems, their contribution to prey breakdown and nutrient cycling within *Darlingtonia* remains poorly characterized[3].

Understanding the roles of the fungal and bacterial residents is important because microbial composition and interactions influence digestion efficiency, nutrient availability to the host plant, and nutrient fluxes within pitcher microhabitats[4]. Clarifying fungal presence and distribution may reveal overlooked pathway of degradation or microbial facilitation that affect plant nutrition and fitness.

I propose using scanning electron microscopy (SEM) to visualize microorganisms in pitcher fluid and on pitcher tissues, with cultured bacterial and fungal isolates from *Darlingtonia* fluid providing morphological references. I examined microorganisms in the hood and lower pitcher to assess their presence within the trap. I hypothesized that yeasts would be present in the lower pitcher alongside bacteria; their presence there would suggest that fungi contribute to decomposition and nutrient cycling within the system.

Micrographs/Figures

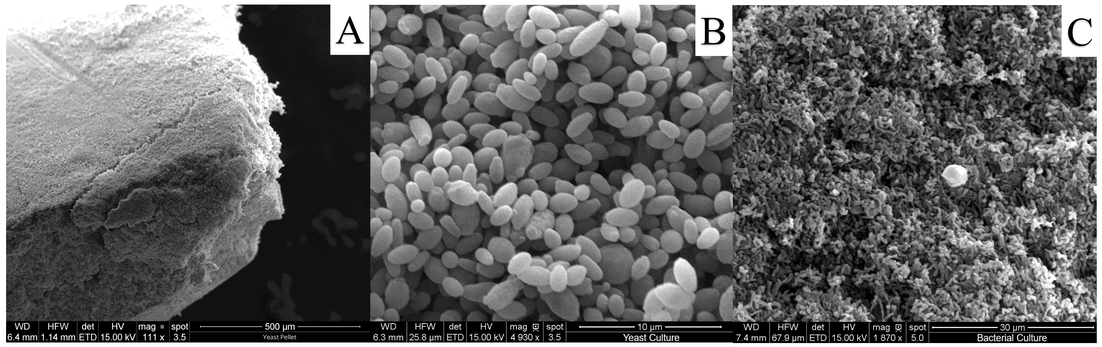

Figure :Cultured microbial isolates from Darlingtonia pitcher fluid. Both fungal and bacterial isolates were grown on agar media and vortexed into a pellet (A). (B-C) Representatives of yeast and bacteria for morphological reference in SEM images.

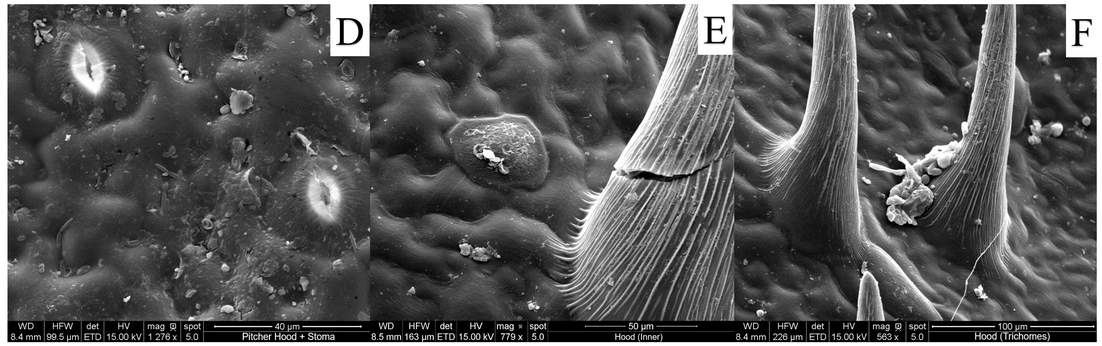

Figure : (D-E) SEM micrographs of microorganisms on the inner portion of Darlingtonia's hood. All three micrographs contain images of what appear to be yeast and bacterial cells.

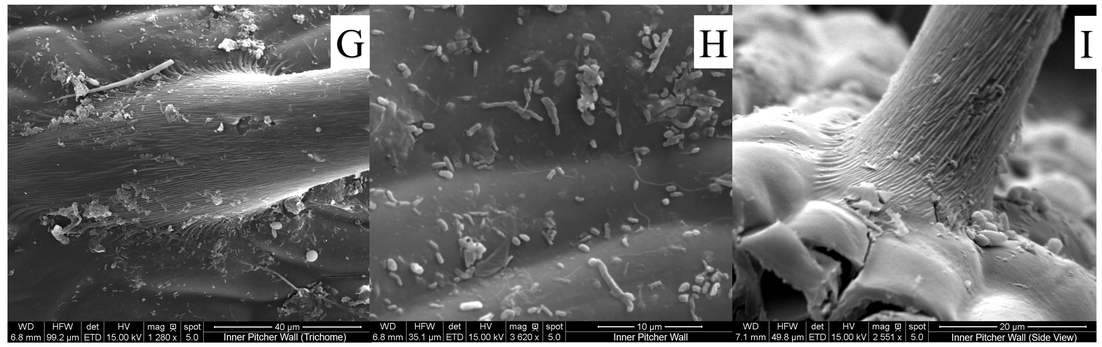

Figure : (G-H) SEM micrographs of microorganisms on the pitcher wall. In image H, there is a close-up of the bacterial cells on the tissue surface.

Methodology

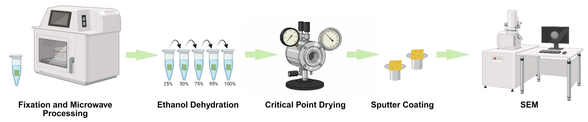

Figure 2: Diagram of the SEM Tissue Processing Procedure.
Image made with BioRender.com

Scanning electron microscopy (SEM) Preparation began with:

- The hood and lower stem were separated and preserved in 200 proof ethanol.
- Small sections (~1 mm × 1 mm) were taken from the hood near the fenestrations, and transverse sections (~0.5 mm thick) were cut from the lower pitcher.
- Pitcher fluid was aseptically extracted, and pure yeast and bacterial colonies were cultured.
- Microbial colonies were collected in water and centrifuged (13,000 rpm, 1 min) to provide a pellet to be used for size and morphology references.
- Samples were fixed in 3% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.0) using microwave-assisted processing, then washed and dehydrated through a graded ethanol series (25%, 50%, 75%, 95%, and 100%, including two final 100% exchanges) to progressively replace water and minimize structural distortion.
- After dehydration, samples underwent critical point drying, where ethanol was exchanged for CO_2 and brought to its critical point to eliminate surface-tension effects and prevent collapse of delicate structures.
- Specimens were mounted onto stubs to expose exterior, interior, and cross-sectional surfaces, sputter-coated with a thin layer of gold, and imaged by SEM.

Results

SEM revealed the presence of both bacteria and yeast-like fungi within *Darlingtonia* pitchers (Fig. A–I). Cultured isolates (Fig. A–C) provided morphological references that aided in distinguishing between bacterial and fungal cells in situ. Microorganisms were observed in both the pitcher hood (Fig. D–F) and the inner pitcher wall (Fig. G–I). In each region, bacterial cells and yeast-like structures were present. Fungal cells were observed in areas where prey is likely to accumulate and undergo decomposition, suggesting a potential role in these processes. However, the presence of microorganisms in the hood may reflect sample handling, as hood and pitcher tissues were stored together in ethanol prior to processing. On the inner pitcher wall, microorganisms were frequently observed associated with the tissue surface (Fig. G–I). Higher-magnification imaging (Fig. H) revealed bacterial cells attached directly to the surface, suggesting close interaction with the pitcher environment.

Conclusion

Yeast-like fungi were observed within *Darlingtonia* pitchers, including in regions associated with prey accumulation and decomposition. These findings suggest that fungi may contribute to prey breakdown alongside bacteria, rather than being incidental members of the system. Together, this supports a more complex view of digestion in Darlingtonia, where multiple microbial groups are likely involved. Further work is needed to determine the functional role of fungi in this system and their contribution to nutrient cycling.

References

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