JOUST
Journal Of Undergraduate Research Students

Isolation and characterization of endophytic fungi in *Dionaea muscipula*

Eva M. Rivera-Meléndez
University of Puerto Rico, PR

Sandra L. Maldonado-Ramírez
University of Puerto Rico, PR

Dionaea muscipula, most commonly known as the Venus flytrap, is a fascinating plant that grows in nutrient-deficient habitats and has evolved to obtain most of its resources by catching insects. Like other plants, the Venus flytrap undergoes photosynthesis, but do they also establish symbiosis with endophytic fungi as other plants do to improve their metabolic activities? We present here a small scale project where we used culture-dependent techniques to isolate fungal endophytes from asymptomatic leaves of *Dionaea muscipula*. All sampled leaves revealed the presence of endophytes, grouped in nine morphotypes, including *Gliocladium sp.*, *Penicillium sp.*, two *Colletotrichum sp.*, four mycelial forms lacking reproductive structures, and one representative form of Mucoromycotina. Mycelial endophytes in *Dionaea muscipula* have not been studied and their roles in the plant are still unknown, but colonization may contribute to host survival, nutrient acquirement and protection.

Introduction

Carnivorous plants evolve various mechanisms to attract, trap and digest insects in order to acquire essential nutrients, especially nitrogen, scarce from the nutrient-deficient soils where they grow (Gao *et al*., 2015). Strategies for prey capture involve one of four mechanisms: adhesive traps, sucking bladder traps, pitfall traps, and snap traps (Kurup *et al*., 2013). Snap trap is the mechanism in *Dionaea muscipula*, the Venus flytrap, which gets its prey through one of the fastest movements among plants (Volkov *et al*., 2008). *Dionaea muscipula* is endemic from coastal wetlands of North Carolina and South Carolina (Gao *et al*., 2015), adapted to tolerate inundation, guarantee vigor of seedlings after fires, and propagate on sandy soils covered by *Sphagnum spp*. (Luken, 2005). The Venus flytrap grows as a rosette, with modified leaves consisting of a lamina and a bilobulated trap packed with digestive glands and trigger hairs in the center, and cilia at the upper margins (Volkov *et al*., 2008) (Figure 1).

Figure 1. Dionaea muscipula, the common Venus flytrap. When stimulated, it triggers hairs that cause the lobes to close, and a cilia overlap, avoiding prey release until digestion.

In order to attract prey, *Dionaea* releases volatile organic compounds such as monoterpenes, sesquiterpenes, alkanes, aldehydes, alcohols, and organic acids (Krewzwieser *et al*., 2013). They also reflect red anthocyanin pigment in the inner surface of the trap (Volkov *et al*., 2008), emit blue fluorescence of 366nm at the inner sides (Kurup *et al*., 2013) and secrete nectar near the cilia base (Krewzwieser *et al*., 2013). Trap closure initiates via mechanostimulation of one or more trigger hairs, which activates ion channels and generates an action potential in 0.3 seconds when the environment temperature is around 36-40°C (Volkov *et al*., 2008).

Evolutionary research suggests the transition of plants from an aquatic environment to land has been achieved by a symbiotic relationship with fungi over 400 million years ago (Singh *et al*., 2011).

Endophytic fungi live asymptomatically within aerial plant tissues and have roles as mutualists, commensals, temporary inhabitants, latent pathogens or latent saprotrophs of the host plant (Redman *et al*., 2001). Also, endophytes impact plant survival by providing mechanisms to interact with the environment and surrounding pathogens. Benefits that endophytic fungi confer include plant biomass increase, drought tolerance, and herbivory protection through chemicals depending on the host and the environmental conditions (Singh *et al*., 2011), as well as help with biotic and abiotic stress tolerance, nutrient acquisition and growth enhancement (Redman *et al*., 2001). The main objective of this research is to characterize for the first time cultivable fungal endophytes from the carnivorous plant Dionaea muscipula using common culture-dependent methods.

Materials and Methods

Plant material

Three leaves from a single individual of *Dionaea muscipula* were sampled for the presence of cultivable endophytic fungi. Sampled leaves were fully turgid and without signs of disease. They were removed by the petiole using a sterile scalpel. Superficial disinfection was performed for each leaf by serial immersions for 10 seconds in 70% isopropyl alcohol, 15% sodium hypochlorite, and distilled water. After disinfection, they were dried with sterile paper towel and processed on a disinfected surface under a laminar flow hood. Using a single edge blade (#10), five segments of approximately 12 mm long were cut from each leaf: two from the trap, one at the apex below the trap, one at the center, and one at the base, near the petiole (Figure 2). Segments were placed in Petri dishes containing Malt Extract Agar (MEA) supplemented with chloramphenicol to inhibit bacterial growth.

Fungal isolation and identification

Petri dishes were incubated for 14-21 days at 25°C until mycelial growth was visible from the borders of the sterilized surface segments. Individual isolations from different morphotypes were made to obtain pure cultures on small sterile MEA dishes and another series on Water Agar (WA). These isolates were also incubated for 14 days at 25°C and characterized by describing macroscopic characteristics- margins, elevation, texture, color, and presence of exudates- of individual colonies. Mycelia with reproductive structures were examined on semi-permanent slides with lactophenol-cotton blue under a light microscope (60X). Frequencies of isolation and percentages were calculated in relation to the total isolates identified as mitosporic fungi, yeast or dimorphic fungi.

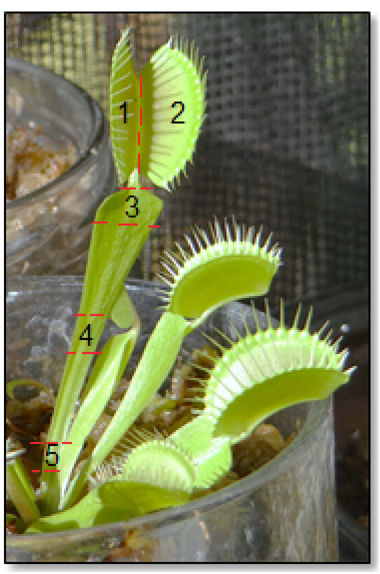

Figure 2. Cut sections of *Dionaea muscipula*. Red lines show the areas from the Venus flytrap cut with a sterile blade. Numbers indicate the segments removed and sampled.

Results

We recovered mycelial growths from all incubated fragments on MEA. Mycelium growing on WA did not grow enough nor mature enough to be characterized. Eight morphotypes were described: two belonging to *Colletotrichum*, one *Gliocladium*, one *Penicillium* and three morphotypes classified as sterile mycelium due to the lack of reproductive structures and the presence of chlamydospores. Another culture, associated to Mucoromycotina, was identified as *Absidia sp*. (Figure 3). Among the endophytes recovered, mycelial fungi were the most frequent (0.75) followed by unidentified yeasts and dimorphic fungi in the same frequency (0.12). Figure 3 shows representative cultures and structures of some of the endophytes identified.

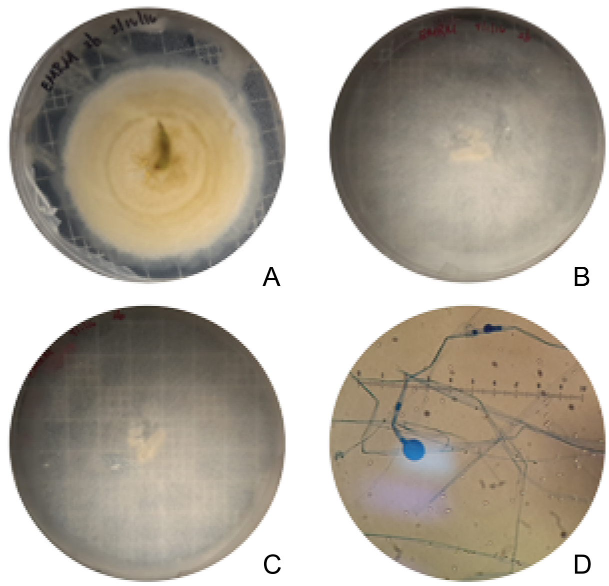

Figure 3. Cultures from *Dionaea muscipula* fragments (A-C) and reproductive structure and spores from *Absidia*, a representative of Mucoromycotina (D).

Discussion

Most of the endophytic fungi previously reported from vascular plants belong to the Ascomycota, but Basidiomycota and former Zygomycota can also be isolated (Higgins *et al*., 2006). From the genus identified, three represent asexual phases of Ascomycetes: *Colletotrichum*, *Penicillium* and *Gliocladium*, while *Absidia*, is a former zygomycete. *Colletotrichum* is widely recognized as a plant pathogen, but is commonly found in asymptomatic plant tissue, as an endophyte. According to other studies, *Colletotrichum* may also affect the way other fungi of the same genus or different pathogens affect the plant (Redman *et al*., 2001).

Colletotrichum and *Penicillium* were found as part of the microbiota in aerial parts of the carnivorous plant *Sarracenia spp*. (Glenn and Bodri, 2012). *Penicillium,* a widely recognized mycotoxin producer, as endophyte can promote plant growth and resistance to hostile environmental conditions (Leitão and Enguita, 2016).

Gliocladium has been commonly identified as an important biocontrol agent against pathogenic fungi by the production of gliotoxin, taxol, and other compounds (Sreekanth *et al*., 2011).

Conclusion

Endophytic fungi can be found in a wide variety of plants, and carnivorous plants are no exception. It is possible that these fungi help the plant survive in its hostile environment. Since carnivorous plants are angiosperms, nonclavicipitaceous fungi were recovered from within Venus flytrap asymptomatic tissues. Nonclavicipitaceous fungi may have the same effect on carnivores as they do on non-carnivore plants, protecting them from predators, assisting in nutrient breakdown and stress tolerance. Further investigations to evaluate more leaves are necessary to determine the exact community of endophytes associated with the Venus flytrap and their roles within the plant tissues. A more comprehensive research is recommended to corroborate these initial findings and to increase the opportunity to isolate more fungi from the Venus flytrap tissues.

References

Gao, P., Loeffler, T., Honsel, A., Kruse,J., Krol, E., Scherzer, S., Kreuzer,I., Bemm,F., Buegger, F., Burzlaff, T., Hedrich, R. and H. Rennenberg, (2015). Integration of trap- and root-derived nitrogen nutrition of carnivorous *Dionaea muscipula.* New Phytologist, 205: p. 1320-1329.

Glenn, A. and M. S. Bodri, (2012). Fungal Endophyte Diversity in *Sarracenia.* PLoS ONE, 7(3): p. e32980.

Higgins, K. L., Arnold, A. E., Miadlikowska, J., Sarvate, S. D. and F. Lutzoni, (2006). Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. Molecular Phylogenetics and Evolution, p. 1-13.

Kurup, R., Johnson, A., Sankar, A., Hussain, A., Sathish, C. and B. Sabulal, (2013). Fluorescent prey traps in carnivorous plants. Plant Biology, 15: p.611-615.

Leitão, A. L. and F. J. Enguita, (2016). Gibberellins in Penicillium strains: Challenges for endophyte-plant host interactions under salinity stress Microbiological Research, 183: p. 8-18.

Luken, J., (2005). Habitats of *Dionaea muscipula* (Venus' Fly Trap), *Droseraceae*, Associated with Carolina Bays. Southeastern Naturalist, 4(4): p.573-584.

Redman, R. S., Dunigan, D. D. and R. J. Rodriguez, (2001). Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytologist, 151: p. 705-716.

Singh, L.P., Gill, S.S. and N. Tuteja, (2011). Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signaling & Behavior, 6(2): p. 175.

Sreekanth, D., Sushim, G. K., Syed, A., Khan, B. M. and A. Ahmad, (2011). Molecular and Morphological Characterization of a Taxol-Producing Endophytic Fungus, *Gliocladium sp*., from Taxus baccata. Mycobiology, 39 (3): p. 151-157.

Volkov, A., Adesina, T., Markin, V. and E. Jovanov, (2008). Kinetics and Mechanism of *Dionaea muscipula* Trap Closing. Plant Physiology, 146: p. 694.