



The endophyte's endophytes: the microbial partners of the endangered plant parasite *Rafflesia* and potential applications in conservation ecology

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ABSTRACT

The “panda of the plant world”, *Rafflesia* is an endangered plant producing the largest flowers in the world. It is also a parasitic endophyte—a plant living inside the vines of its host plant, *Tetrastigma*. Endemic to the dwindling forests of Southeast Asia, propagation attempts to conserve *Rafflesia* have been largely unsuccessful. In this study, we explored the bacteria living inside the parasite, *Rafflesia*'s own endophytes. Plants harbor a diverse microbiome—bacterial endophytes residing within plant tissues that may offer mutualistic benefits to their plant partners such as enhancement of growth and pathogen resistance. The goal is to characterize *Rafflesia*'s bacterial endophytes and understand from the literature their microbial ecology, as they may be important in facilitating and sustaining *Rafflesia* in its host. We outourced sequencing of the microbiome of *Rafflesia* seeds and its host. A genus-level phylogeny of the bacterial endophyte community in all samples was reconstructed with bacterial proportions for each sample mapped on the phylogeny. We predicted that the *Rafflesia* seed and the infected host would have a more similar bacterial phylogenetic composition compared to the uninfected host even though host infection has not yet happened since we are sampling the *Rafflesia* seed. We found that multiple genera in the bacterial phyla Firmicutes and Bacteroidetes were uniquely shared between the *Rafflesia* seed and its infected host suggesting that *Rafflesia* may alter host microbiome upon infection. These bacterial endophytes could serve as bioinoculants that may have applications in the ex situ propagation and conservation of *Rafflesia*.

INTRODUCTION

- Rafflesia* has giant (Fig. 1), odoriferous, parasitic flowers with no stems, roots nor leaves—defying what is known of “typical” plants. It is also an endophyte, living inside the vine *Tetrastigma* (Fig. 2) and only emerging to flower.
- Rafflesia* is one of the only 2 plants in the world to have completely lost its plastid genome, the set of genes that make plants photosynthetic and define what they are (Molina et al. 2014)
- Techniques to propagate *Rafflesia* outside its natural habitat have been unsuccessful. Since 2015, Molina et al. (2017) have been transporting viable specimens of the genus and its host species for cultivation in the US Botanic Garden (USBG) in Washington, DC, without success.
- Plants, including *Rafflesia*, harbor a diverse microbiome—bacterial endophytes residing within plant tissues that may offer mutualistic benefits to their plant partners such as enhancement of growth and pathogen resistance. Interestingly, plants have the ability to “select” and “recruit” endophytes into their microbiome (Santoyo et al. 2016)
- In this project, we sought to learn more about *Rafflesia*'s seed microbiome—the endophytes of the endophyte itself. Characterizing the *Rafflesia* seed bacterial microbiome may hold clues on mutualistic microbial ecological interactions (e.g., bacterial production of metabolites that promote *Rafflesia* growth and/or suppression of host defenses), which may have applications in *Rafflesia* propagation and conservation.

MATERIALS & METHODS

- The Philippine endemic *Rafflesia speciosa* only parasitizes host roots. Twelve samples including seeds of *R. speciosa* (4, R), root cuttings of *Rafflesia*-infected host *Tetrastigma harmandii* (6, I) and of non-infected host roots (2, N) were collected, surface-sterilized with 2% sodium hypochlorite, rinsed and sent by J. Molina for DNA extraction and 16S microbiome sequencing and metagenomic analyses to Zymo Research (Irvine, CA).
- Resulting bacterial sequences from Zymo's metagenomic analyses were downloaded by UBRP students, aligned and phylogenetically analyzed in Geneious Prime (Biomatters, Ltd). Bacterial abundance information were mapped on the resulting phylogeny using ItoL (Interactive Tree of Life, Letunic and Bork, 2021).
- We hypothesized that the *Rafflesia* seed (R) would have a similar microbiome composition as the infected *Tetrastigma* host (I), but quite different from the non-infected *Tetrastigma* (N).
- Uniquely shared bacterial genera between the *Rafflesia* seed and the infected *Tetrastigma* (RI, blue bars in Fig. 3) were then researched in the literature for ecological functions and discussed.



Fig. 1. Blooming flower of *Rafflesia speciosa*, one of the “medium-sized” *Rafflesia* species at about 45 cm, with mentor, J. Molina, to demonstrate enormous size of flower. Photo taken in the rainforests of Miagao, Iloilo, Philippines.

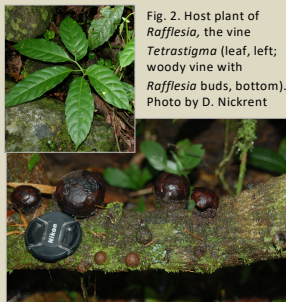


Fig. 2. Host plant of *Rafflesia*, the vine *Tetrastigma* (leaf, left; woody vine with *Rafflesia* buds, bottom). Photo by D. Nickrent

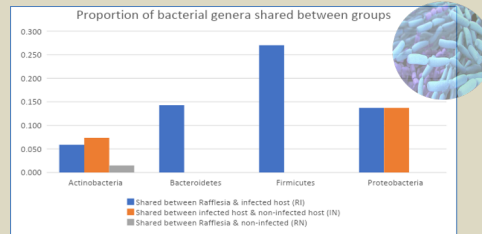


Fig. 3. Bar chart to show number of genera per phylum shared across samples (RI, IN, RN). There are more genera shared between the *Rafflesia* seed and the infected host (RI, blue).

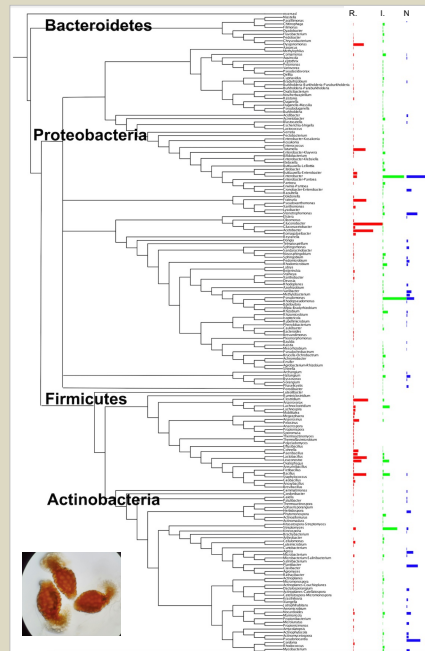


Fig. 4. Phylogeny with bacterial proportions per genus mapped to the right as colored bars with length proportional to abundance. R (red): proportion in *Rafflesia* seed; I (green): infected host; N (blue): non-infected host. Many genera in Firmicutes have similar abundance between R and I (RI, boxed). Inset: *Rafflesia* seeds magnified 100x, each seed is tiny, c. 1 mm

RESULTS & DISCUSSION

- Phylum Firmicutes and phylum Bacteroidetes have > 25% genera and almost 15% genera, respectively, shared between the *Rafflesia* seed and infected host (RI, Fig. 3), but none shared between IN and RN. In Actinobacteria and Proteobacteria, this pattern is absent.
- Mapping bacterial abundance by genus on the phylogeny (Fig. 4), we were able to determine specific bacteria that have similar abundances across samples. In phylum Firmicutes, we see similar bacterial abundances for many genera between the *Rafflesia* seed and the infected host (RI). This suggests that *Rafflesia* seems to alter host endophytic composition of Firmicutes genera similar to its own, presumably via horizontal transfer upon infection.
- In Firmicutes, bacterial genera shared between RI (but not between IN nor RN) included lactic-acid producing *Enterococcus*, *Lactobacillus*, and *Leuconostoc* which were found to have plant-growth promoting properties including increased phosphate assimilation and pathogen resistance (Khalaf and Raizada 2016; Liu et al. 2019; Msimbira and Smith 2020). *Enterococcus* can also digest cellulose which could allow *Rafflesia* to break down host tissue for infection (Ramsey et al. 2014). Another genus shared was *Paenabacillus* which helps with significant root development (Ulrich et al. 2008), which *Rafflesia* may be taking advantage to enhance host root growth. *Clostridium* can fix nitrogen making this nutrient more available to *Rafflesia* and its host (Figueiredo et al. 2020).
- In phylum Bacteroidetes, *Chryseobacterium*, known to produce proteolytic enzymes (Dogan and Taskin, 2021) was shared between RI as well as another unidentified genus. *Chryseobacterium* may be used by *Rafflesia* to break down proteins in *Tetrastigma* for nutrient absorption.
- In general, the various bacteria shared between RI (but not between IN nor RN) were found to have plant-growth promoting properties including enhancing nutrient uptake, pathogen resistance, as well as production of solubilizing enzymes, which presumably, are exploited by *Rafflesia* to facilitate infection of its host.
- Other studies have found similar results regarding the microbiome of parasitic plants with endophytic bacterial strains enhancing nutrient uptake for the plant parasite (Felestrino et al., 2017). Therefore, it could be that microbial partners are involved in regulating *Rafflesia*'s life cycle (Wicaksono et al. 2020).
- These bacterial endophytes with plant-growth promoting properties can serve as bioinoculants, which may facilitate propagation and conservation of *Rafflesia*, the “panda of the plant world”.

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