Genomic analysis shows bacterial community shifts in *Exaiptasia pallida* between environments.

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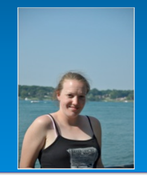


Abstract

The sea anemone Ex*aiptasia pallida* is a model system for studying the symbioses between cnidarians and microbial organisms; and for investigating the physiological response to environmental stressors. These studies are conducted in laboratory conditions using anemones that have been cultured for many years. However, it remains unknown if the bacterial symbionts associated with the laboratory sea anemones remains similar to those found in natural environments. Therefore, it is critical to determine if lab cultured anemones contain comparable microbiomes or associated microbes to those in natural environments. In this study, the bacterial communities associated with lab-cultured anemones were compared with anemones sampled from the natural environment in the Florida Keys (Long Key) and from a pet store. DNA extractions were conducted on three anemones from each source. The identification of bacteria was performed using 454 pyrosequencing of the V1-4 region of 16S rRNA. Results showed a lower variation of bacterial communities within anemones from the wild and the laboratory compared to those identified in anemones from the aquarium (pet) store. Firmicutes mainly represented the bacterial communities of wild anemones while lab-reared anemones were mostly composed of Proteobacteria. These findings suggest that the environment is a selective force for structuring the bacteria community that resides within *A. pallida*.

Aims

1. Determine the composition and structure of the bacterial community associated with *E. pallida* inhabiting different environments (Wild versus captivity).
2. Determine whether the anemone-associated bacterial assemblages correlate with the composition of the symbiotic dinoflagellate, *Symbiodinium* living within the sea anemone.

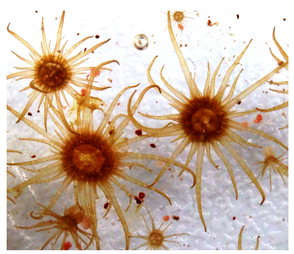

Figure 1. Clonal culture of *E. pallida*, implemented as a model system to study cnidarian symbioses.

Methods

1. **Sample Collection:** *E. pallida* were collected from three different locations in triplicate. The samples were collected from a six-year laboratory population, a pet store, and from a natural population at Long Key (Florida Keys).
2. **DNA Extraction and 454 Pyrosequencing of V1-V4 16S rRNA:** DNA was extracted using the MOBIO Power Soil DNA Isolation kit (MOBIO, Carlsbad, CA) using standard manufacturers protocols. The V1-V4 region of the 16S rDNA was amplified and pyrosequenced at the sequencing facility of Mr. DNA (Molecular Research LP).
3. ***Analysis of Microbial community:*** Non-metric multidimentional scaling (nMDS) analysis was performed using relative abundances of the Operational Taxonomic Unit resolved after the clustering of chimera-filtered sequenced reads. Taxonomic classification of the OTUs was performed using RDP Naïve Bayesian Classifier.
4. ***Symbiodinium Identification:*** The cpr23S and nr28S regions of *Symbiodinium* DNA were amplified and sequenced. Subsequent sequence analysis allowed for the identification of *Symbiodinium* clades.

Results

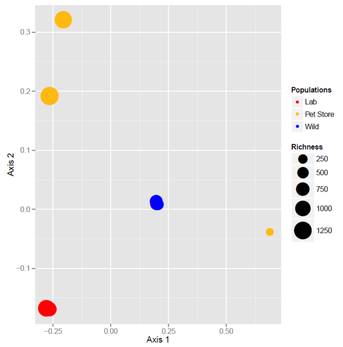

Figure 2. (Left) Nonmetric multidimensional scaling (nMDS) ordination using V1-V4-16S rRNA OTUs of microbial assemblages associated with *E. pallida*.

- **The bacterial community structure differed across environments, detecting a high variability among the samples from the pet store.**

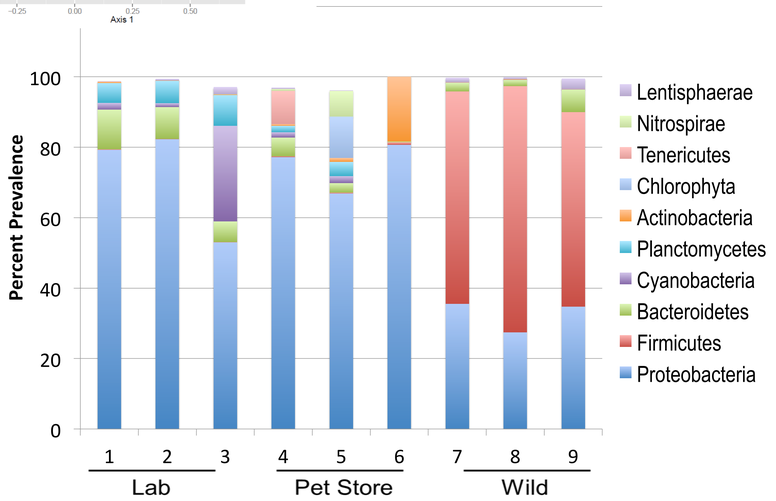

Figure 3. (Above) Prevalence and distribution of bacterial phyla identified in the microbial community associated with *E. pallida* collected from different locations.

- **Host-associated bacterial community from the lab and pet store was dominated by Proteobacteria, unlike those from the wild, which were dominated by Firmicutes.**

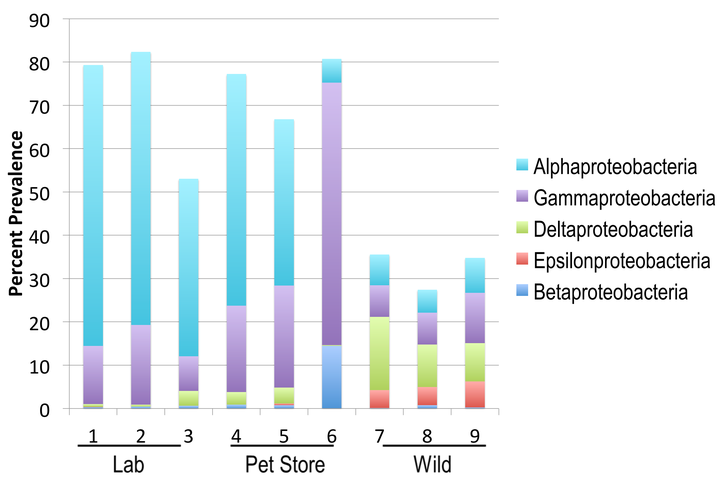

Figure 4. (Above) Prevalence and distribution of bacterial classes within the Proteobacteria phylum identified in the microbial community associated with *E. pallida* collected from different locations.

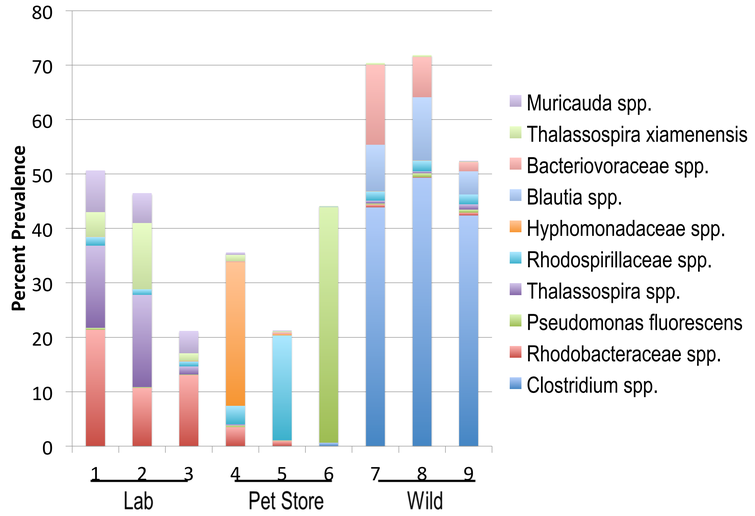

Figure 5. Prevalence of the ten most abundant bacteria found in association with *E. pallida* collected from the different locations.

- **While the most abundant bacterial species were different among environments, species dominance was conserved within each environment except from those anemones from the pet store. In this case, each anemone had a very different set of dominant bacterial species.**

Table 1. Clade composition of *Symbiodinium* associated with *E. pallida* collected from different locations.

***Symbiodinium* Clades**			
	Clade A	Clade B	Clade C
Lab (Captivity)	3	0	0
Pet Store	1	1	1
Wild	2	1	0

Conclusions

- *E. pallida* shows a low specificity regarding the composition of bacteria species it associates with.
- The higher variability of the bacterial community structure detected in anemones from the pet store suggests that these hosts have been exposed and engaged with a myriad of bacterial species from co-existing invertebrates originating from diverse locations.
- The distinct bacterial composition of those anemones kept in laboratory captivity in comparison to those from natural environments in the Florida Keys raises caution as to using this model system reared in the lab to study bacterial symbionts.
- Further studies are needed to determine if the environment or the host is the selective force for the bacterial community make up.
- No correlation was found between bacterial community assemblages and the composition of *Symbiodinium.*