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Data integration to define environmental and host factors impacting shrimp microbiota.

Instituto de Biotecnología

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Introduction

The microbiota plays essential roles in the development and physiology of their host, such as preventing growth of pathogenic bacteria, modulating the immune response, and regulating metabolic processes.

Recent advances in high-throughput sequencing of the ribosome subunit 16S (16S rRNA gene), plus the importance of shrimp for commercial distribution, have increased the interest in characterizing the bacterial communities of this organism and its habitat. However, most of the studies use different experimental and bioinformatics protocols, making it difficult to compare their results.

The technical and bioinformatics differences include the selection of 16S rRNA hypervariable region, the use of different PCR primers for the same hypervariable region, different DNA extraction protocols database selection for taxonomy assignment and different clustering algorithms. For 16S rRNA amplicon studies, these biases can be minimized analyzing the data with similar bioinformatics methods.

We performed a meta-analysis of shrimp microbiota using all available high-throughput 16S rRNA sequencing data and the same bioinformatics protocol to explore the impact that biological factors such as habitat, farm, laboratory, organ, developmental stage, disease, and diet, have on the microbiota structure and composition.

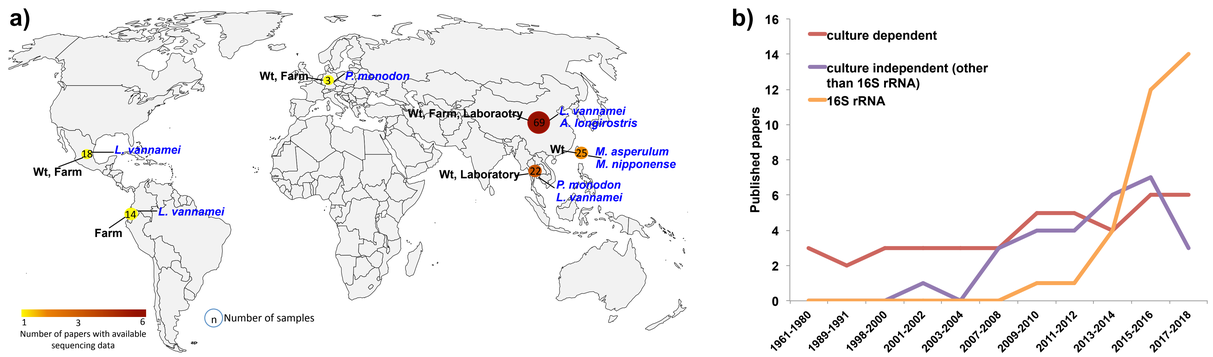

Figure 1. a) Geographic distribution of all studies with publically available sequencing data. The shrimp species, lifestyle condition, and the number of sequenced samples are show for the countries. **b)** Year distribution of all studies grouped into the use of culture-dependent, culture-independent or 16S rRNA gene sequencing.

Methods

All available studies related to shrimp microbiota were identified by a systematic search on SCOPUS on February 1 of 2018. To be considered, the studies had to include: (i) freely available 16S rRNA sequencing data; and (ii) sequencing data correctly separated by sample type. This process led to 16 studies that grouped 199 samples (Figure 1).

All sequences were filtered maintaining a minimum quality of Q20, a minimum length of 90 bp. The remaining sequences partitioned by sample, were clustered at 97% identity into operational taxonomic units (OTUs) against the Greengenes database (version 13_8) using UCLUST in QIIME 1.9.1.

We eliminated the OTUs represented by a single read (singleton) or with a frequency 0.005 for further analyses. Finally, all alpha indices and analysis of similitud (ANOSIM) were calculated with QIIME 1.9.1.

Results

A one-way analysis of similarity (ANOSIM) revealed that the most significant impact on the microbiota structure was by technical factors: study (R= 0.984, p=0.001), amplification primers (R=0.846, p=0.001) and hypervariable region (R=0.817, p=0:001). The fourth most important factor was the marine and freshwater environment (R=0.561; p=0:001) **(Figure 2).** We considered this the main biological factor that drives the shrimp microbiota.

In the basis of the main separation observed between marine and freshwater, we performed all further analyses only considering the 126 marine samples. Here, the ANOSIM showed that lifestyle (R=0.341, p=0.001), organ (R=0.279, p=0:001) and developmental stage (R=0,240, P=0:001) were the main biological factors shaping the shrimp microbiota.

Furher, the most diverse samples were from a wild lifestyle, adults, stool and wild-type diets. And, as noted in other studies, samples from diseased shrimps showed lower diversity **(Figure 3).**

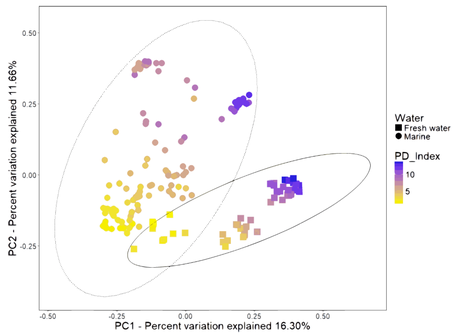

Figure 2. Unweighted principal coordinate analysis (PCoA) of UniFrac distances for samples tagged by marine or freshwater origin. The color gradient shows the Phylogenetic Diversity index (PD).

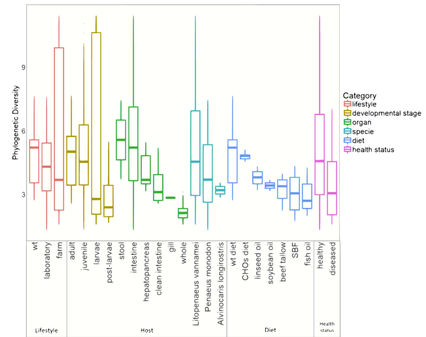

Figure 3. The Boxplots indicated the phylogenetic diversity index (PD) for all samples grouped by categories.

Finally, a linear discriminant effect size (LEfSe) analysis showed differentially abundant genera among the different categories **(Figure 4).** For example, Enterobacter was differentially abundant in farm samples, while Bacillus appeared in wild-type samples. Notably, these both genera are known for their probiotic properties.

On the other hand, Vibrio, which has some species that act as oportunistic pathogens was differentially abundant in adult and clean intestine samples.

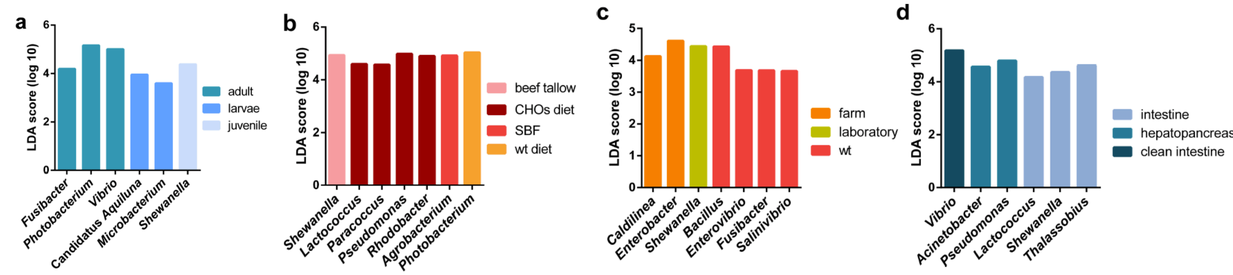

Figure 4. LEfSE results of enriched genera for marine samples in different categories.

Conclusions

Aside from technical factors, our study highlights that biological factors, such as, lifestyle, organ and developmental stage significantly shape the structure of the shrimp microbiota. Further studies are needed for a better understanding of the role that these factors play, including a more significant number of samples and other shrimp species.

Reference: Cornejo-Granados et al. (2018), A meta-analysis reveals the environmental and host factors shaping the structure and function of the shrimp microbiota. PeerJ 6:e5382; DOI 10.7717/peerj.5382