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Revealing the uncultured bacteria inside the giant snail Achatina fulica

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The giant snail *Achatina fulica* is an agricultural pest whose biotechnological potential has been largely untested. Here, the composition of the microbial population within the crop of this invasive land snail, as well as key genes involved in various biochemical pathways, have been explored. In a high-throughput approach, 318 Mbp of 454-Titanium shotgun metagenomic sequencing data were obtained. The predominant bacterial phylum found was *Proteobacteria*, followed by *Bacteroidetes* and *Firmicutes*. *Viruses*, *Fungi*, and *Archaea* were present to lesser extents. The functional analysis reveals a variety of microbial genes that could assist the host in the degradation of recalcitrant lignocellulose, detoxification of xenobiotics, and synthesis of essential amino acids and vitamins, contributing to the adaptability and wide-ranging diet of this snail. More than 2,700 genes encoding glycoside hydrolase (GH) domains and carbohydrate-binding modules were detected. When we compared GH profiles, we found an abundance of sequences coding for oligosaccharide-degrading enzymes (36%), very similar to those from wallabies and giant pandas, as well as many novel cellulase and hemicellulase coding sequences, which points to this model as a remarkable potential source of enzymes for the biofuel industry. Furthermore, this work is a major step toward the understanding of the unique genetic profile of the land snail holobiont.

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Phylogenetic identification and characterization of microbial diversity in Moroccan and Portuguese rivers using a metagenomics approach

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The microbial communities in fresh and mine contaminated water has an important role in the biogeochemical cycling of nutriments and the clean-up of pollution. Mine sites are an example of extreme environment that contains a large number of diverse and specific bacteria. only 10% of these microbes can be studied by classical culture. Metagenomics analysis gives the opportunity to study better the functioning of these natural ecosystems.

The aim of this project is to exploit the metagenomics approach for exploration of microbial diversity of three Moroccan rivers (Sebou, Moulouya end Oum Errabiaa) We characterize and compare microbial community composition and structure in these populations using bioinformatics and computational tools to identify taxonomic groups and genes (biomarkers) to suggest which environmental factors influence the selection of microorganisms.

Metagenomics data generated by this work are used to make comparison with Portuguese ecosystems to suggest which environmental factors influence microbial diversity in this site and the selection of microorganisms that might have an impact on the environmental and human health.

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Fingerprinting microbial communities through flow cytometry and Raman spectroscopy

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Natural and engineered communities can be more accurately characterized –and therefore managed- with single-cell technologies. Raman spectroscopy is an emerging tool for single-cell analysis. It is capable of making a fingerprint for a single bacteria, using the information of the (bio)molecules that are present in the cell. This fingerprint can be acquired without using a dye, and without damaging the sample, that can be further used.

As Raman spectroscopy becomes more widely used in microbial ecology, there is a need to ameliorate the standardization of data acquisition. Our group has demonstrated how different sources of noise due to improper sample manipulation can result in artificial subpopulations. Therefore, we have proposed a metadata aid to report measurements. We are also developing the R package "MicroRaman" to assist users in the analysis of microbial Raman data. In this way, we hope to improve experimental reproducibility and sharing of spectral data between research groups.

We compared Raman spectroscopy to another technology for microbial fingerprinting: flow cytometry. This single-cell technology is lower in resolution, but much more high-throughput than Raman spectroscopy. To compare their capacity to identify bacterial phenotypes, we harvested *E. coli* in different growth stages and analyzed them using both techniques. Our results indicate that flow cytometry allows to quantify shifts in the community fingerprint at the population level, while Raman spectra contained sufficient resolution to detect separated subpopulations at the single-cell level.

Phenotypic heterogeneity allows bacteria to adapt to a changing environment, as well as to divide the labor within the community. As both Raman spectroscopy and flow cytometry allow to quantitatively measure phenotypic heterogeneity, these technologies may become an important tool to link bacterial fingerprints with functionality. Ultimately, these tools might allow to steer microbial communities to their most optimal phenotype.