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UNIVERSIDADE DO ALGARVE
FACULDADE DE CIÊNCIAS E TECNOLOGIA

Microbial Community Profiling of Algarve (Portugal) Marine Coast Line

Paulo Ricardo Gonçalves Cerqueira, 54492

Professor Leonor Faleiro

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Introduction

Marine microbial communities are consisted of bacteria, archaea, unicellular eukaryotes, fungi and viruses, which have complex roles in biogeochemical processes of carbon, nitrogen, phosphorus, sulphur and iron cycles (Aylward *et al.*, 2015; Fuhrman *et al.*, 2015; Galand, 2018). Such microorganisms are phototrophs that uses sunlight as a source of energy, chemotrophs that oxidizes chemical substrates to obtain energy or heterotrophs that needs to consume organic carbon to obtain energy (Fuhrman *et al*., 2015). Within phototrophs and chemotrophs, we have photoautotrophs and chemoautotrophs that uses carbon dioxide as a biomass source, producing organic matter, which are used by photoheterotrophs and chemoheterotrophs to obtain its biomass (Fuhrman *et al*., 2015). Altogether, those microbial communities are a principal component of the food web involved in the recycling and flux of dissolved organic matter (DOM) (Simon *et al.*, 2014; Fuhrman *et al.*, 2015). Then, establishing a microbial community profile involves a complex analysis of 16S rRNA, 18S rRNA and ITS genes based on the choice of a high throughput sequencing (HTS) technology tool to provide accurate, rapid and cost efficient analysis of the marine environment (Bourlat *et al*., 2013; Aylagas *et al*., 2017;). Quoting Delong & Karl (2005), the understanding of ocean processes are needed to place the emergent genomic-based knowledge of marine microorganisms in the context of biogeochemical cycles of carbon, nitrogen and phosphorus. Those microbial communities are intra and interspecific correlated and they compete for nutrients; symbiotic relations are established and it is important to account for the entire potential and role of such interactions with the environment (Konopka, 2009; Aylward *et al*., 2015; Aylagas *et al*., 2017; Galand, 2018; Zhou *et al*., 2018).

In an ecological point of view, the limiting nutrient could define the composition of a microbial community (Delong & Karl, 2005), as well as, identifying the flux of those nutrients and what are the mechanisms that regulate the biogeochemical processes (Falkowski, 2012; Simon *et al.*, 2014). For example, the productivity of the oceans depends on the biogeochemical transformations by microorganisms, which includes nitrogen fixation, denitrification and assimilation, anaerobic ammonia oxidation, sulfate reduction, methanogenesis, iron and metal reduction reactions (Cycling & Paerl, 1996; Zehr & Kudela, 2011). Another example, is the variety of organic and amino acids, peptides, lipopolysaccharides, carbohydrates and alkaloids excreted by cyanobacteria and eukaryotic phytoplankton (Cycling & Paerl, 1996).

As discussed in Nogales *et al.* (2010), the increase of nutrients, the introduction of xenobiotics into the environment and the pollution by organic chemicals and heavy metals changed the community composition and increased cell number, diversity and temporal variability, which evidenced environmental modifications through anthropogenic pressures. In Aylagas *et al.* (2017), it is also observed a significant correlation between the *Desulfobacteraceae* abundance with the increase of organic matter content in marine environment by direct discharges, runoff from land or river discharges and, in Zhou *et al.* (2018), it was demonstrated a relation between the bacterial community and its shift during a dinoflagellate bloom, supporting the microbial relations.

Microbial communities are driven by different biologic and non-biologic properties of the environment (Fuhrman *et al*., 2015). Then, different timescales, such as hours, days, weeks, months and seasons are needed to address what could happen to microbial composition dynamics (Azam & Graf, 1983; Fuhrman *et al*., 2015). To understand the microbial shifting along the marine coast line of Algarve, environmental factors, such as temperature, salinity, DOM, dissolved oxygen (DO) in water, pH, water currents and its chemical composition, sedimentology and anthropogenic pressures are needed to be accounted to characterize the whole paradox of microbial community dynamism (Azam & Graf, 1983; Bo *et al.*, 2009; Galand, 2018; Lv *et al*., 2016; Zhou *et al*., 2018). The main objective of the work is to establish the microbial community profile of the Algarve marine coast in order to understand the population dynamics along the coast and during different timescales.

Materials and Methods

2.1. *Sample Collection*

During different campaigns, the water samples will be collected aside the coast line at a distance between 200-300 meters. The sediment sampling in different beaches will be collected between tides using bags. Surface water will be collected with a bucket and a 10-L Niskin bottles mounted to a CTD (conductivity-temperature-depth) rosette will be used to collect 1-10 m, 10-30 m and 30-60 m depth waters. All microorganisms present in water column will be collected by filtration of a volume varying 1-6 liters, using specific filters and then preserved and stored at -80 °C to further analysis as described

(Smith *et al.*, 2010). Mirone software will be used for map construction. Sampling coast location is indicated in the map in **Fig. 1**.

2.2. *Environmental Data Processing*

2.2.1 *Multiparameter Measurements*

Temperature, salinity, pH, DOM, DO, will be measured with a multiparametric probe. Waves and weather conditions will be accessed at the IPMA (Instituto Português do Mar e da Atmosfera) and Tide Model Driver Toolbox in Matlab will be used to predict the tides at the moment of each campaign.

2.2.2 *Chemical Measurements*

The samples will be taken to the laboratory in order to assess the chemical properties following (Il, 1972) of the water column according to: chlorophyll *a* (*chl a*), suspended solids (SS) and nutrients nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}). HPLC (high performance liquid chromatography) will be used for photosynthetic pigment analysis by filtering 100-300 mL of water onto a specific filter and calculated as described (Herfort, Peterson, Mccue, Crump, et al., 2011a; Herfort, Peterson, Mccue, & Zuber, 2011b; Smith et al., 2013).

2.2.3 *Sedimentology*

1 kg of sediment will be collected and placed in bags correctly labeled. Afterwards, the grain size will be analyzed by dry sieving technique and organic matter will be analyzed by ignition at 450 °C during 6h (Aylagas et al., 2017).

2.3. *Microbial Community Analysis*

2.3.1 *DNA extraction, Amplification and Sequencing*

The following processes will be based in whole genome sequencing. DNA from soil samples will be isolated using DNeasy PowerSoil Pro Kit from QIAGEN and the DNA from water samples will be extracted using DNeasy PowerWater Kit from QIAGEN. All the methods will be according to manufacturer's instructions.

Shotgun sequencing will be used, based on Singer *et al*. (2016), to obtained both soil and water samples using PacBio RSII platform. After, the quality filtering and error correction of PacBio sequences will be performed as described (Singer *et al*., 2016).

2.3.2 *Taxonomic Profiling Analysis*

For the taxonomic profiling comparison, the MEGAN and MetaCluster software will be used for high-throughput sequencing reads as described (Wang *et al.*, 2014) to obtain the microbial abundaces. LEfSe will be used for identification of taxonomic biomarkers.

2.3.3 *Metabolic Profiling Analysis*

In order to obtain metabolic profiles, BLASTX will be applied against the KEGG database. Then, as described in Larsen *et al.* (2011), PRMT will be used to predict the metabolic turnover and LEfSe will be used for identification of taxonomic biomarkers.

Expected Results and Conclusion



Fig. 1 - Map of Algarve's Coast Line. Both barlavento side and sotavento side are represented to show the sampling region along the coast.

The first approach of the study is to identify the main differences along the coast of Algarve and see if there is any difference in the composition of the communities, as well as, for its alpha diversity, abundance and evenness of each sample.

All the sampling locations are going to be annotated with a specific GPS location. After, a map will be constructed with the sampling points and it will be attributed to each point the communities showing its abundance, composition and diversity in order to give a direct comparison between the points. It is expected that it will be possible to see differences in the coast depending on several biological and non-biological factors.

Rivers are good carriers of nutrients and sediments to the ocean. Excess of nutrients are a consequence of anthropogenic pressures, e.g., the usage of pesticides in agriculture that are washed by runoff of surface waters into the rivers, and it could be possible to see the differences in microbial composition according to different limiting nutrients and different geographical locations and physicochemical compositions of the environment samples, which means that near effluents could be different from dislocated zones. It could be also possible to associate seasonal periods and upwellings events with different abundances and richness of the microbial community.

A secondary objective of this work is the establishment of a scale of coast quality according to microbial composition and see if there is impact on population's health and economics. In order to fulfil the objectives, a construction of a database would be of interest to compile all the obtained metadata to establish a correlation between environment and socioeconomic problems.

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