Title: ISOLATION AND IDENTIFICATION OF ANAEROBIC BACTERIA ISOLATED FROM MARINE SPONGES

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Marine sponges are sessile and filter feeding organisms. They host a lot of microorganisms that can make up to 40% of their body mass. From them, it is possible to characterize a wide variety of bioactive compounds, with the most diverse applications. Interestingly, several compounds isolated from sponges have a striking resemblance to microbial metabolites, suggesting that their microbiota is involved in the production of these substances. Therefore, the potential of these organisms and their associated microbiota as a source of new bioactive compounds is very promising. Given the bacterial diversity associated with marine sponges and their biotechnological importance, this study aims to isolate and identify anaerobic bacteria in marine sponges. Samples of four sympatric morphs of *Plakina* sp. sponge were collected on the coast of Cabo Frio, RJ on May, 2015. The specimens were manually collected from the individuals (sponges) attached to rocks. Then, each sponge sample was placed in thioglycollate broth with resazurin and incubated at ambient temperature in screw top 2.5 L anaerobic jars containing one sachet of anaerobic atmosphere generator. Once back at the lab, all samples were immediately transferred to an anaerobic chamber. The bacteria associated with sponges were obtained by maceration of each specimen, then each extract was diluted serially (10^{-1} - 10^{-5}). Each dilution was spread in four different culture media: BHI, BHI diluted 10x, BHI dissolved in seawater or Marine -agar. The plates were maintained anaerobically at room temperature for 7 days and examined daily for growth and colony morphology. After the incubation period, the colony forming units (CFUs) were selected according to the morphological characteristics. These CFUs were plated in duplicate on BHI agar and incubated under two different conditions: under anaerobic and aerobic conditions. Strains, which grew only under anaerobic condition, were stored at -80 °C in BHI broth with 30% glycerol. Among 92 CFUs selected, 60 were strict anaerobes. Anaerobic strains will be identified by 16S rDNA sequencing and their production of bioactive substances will be investigated. The isolation and cultivation of anaerobic bacteria increases the potential for bioprospecting goals of biologically active compounds, in addition to emphasizing the importance of anaerobic bacteria in the interaction between sponge and microbiota associated to the survival of marine sponge.

Key-words: anaerobic culture, *Plakina* sp., marine sponge

Agência Fomento: CNPq, FAPERJ e PIBIC-UFRJ