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BIOPROSPECTING MARINE MICROORGANISMS AIMING THE DISCOVERY OF NEW BIOACTIVE NATURAL PRODUCTS FOR DRUG DISCOVERY

Luciane A. C. Tonon[1], Patrícia Ballone[1], Rafael de Felício[1], Arthur Z. N. Fernandes[1], Marcelo Brocchi[2], Marcus A. C. da Silva[3], André O. S. Lima[3], Daniela B. B. Trivella[1].

[1]Brazilian Biosciences National Laboratory – National Center for Research in Energy and Materials, Campinas, Brazil; [2]Departamento de Genética, Evolução e Bioagentes - IB – UNICAMP, Campinas. Brazil [3]Centro de Ciências Tecnológicas da Terra e do Mar, Universidade do Vale do Itajaí, Itajaí, Brazil; luciane.chimetto@lnbio.cnpem.br.

Microorganisms of millions species exist in every corner of the Earth, and form a dynamic genetic reservoir [1]. However, expressing the full biosynthetic capacity of these microorganisms under laboratory conditions is challenge. For example, only 1% of microorganisms can growth using traditional cultivation methods, and about 90% of their genetic capacity is hidden in laboratory cultures. Therefore, it is expected that a significant number of microbial natural products are to be discovered. Thus, developing effective strategies to exploit new secondary metabolites from microbial sources is promising to prompt drug discovery efforts [2]. Based on the proposal of discovering new bioactive molecules we have investigated two different bacteria collections, one composed of strains isolated from deep sea and, the other, of strains isolated from different cnidarians host in Brazil. Together, these culture collections preserve around 1,000 bacteria strains. Large fractions of these strains were previously identified by 16rRNA gene sequence and in some cases, whole genomes were available. A set of 40 microorganisms representing different phyla (Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes) were initially selected. The available genomes were used to analyze the presence of biosynthetic gene clusters using bioinformatic tools (antiSMASH) [3]. The analyses suggested 16 gene clusters involved in secondary metabolism, 5 of them displaying up to 78% homology with known clusters - including NRPS, PKS and mixed biosynthetic gene clusters - and 11 with low homologies, indicating new clusters. Selected strains were grew in plates with solid culture medium and were submitted to elicitation tests under different stress conditions. The attempt was to stimulate and modulate activation of the secondary metabolism, and some of the chemical elicitors were selected from genomic mining analysis. Strains able to grow under the stressful conditions were therefore screened and analyzed in liquid medium. Crude extracts were prepared by solid phase extraction and fractionated using preparative reverse-phase LC-UV. These fractions were further analyzed by analytical LC-MS and revealed the presence of 1-15 chemical substances each. A pre-fractionated HTS chemical library was assembled using the prepared fractions and evaluated for the presence of proteasome inhibitors - a validated target against cancer. This first screening allowed us to detect a hit from *Erythrobacteraceae* family acting as a reversible inhibitor of the proteasome. We are now carrying out other biological assays searching for new bioactive natural products. As perspective, we wish increase the number of marine microbial samples investigated in the context of natural products drug discovery.

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