



MARINE MICRORGANISMS RECOVERED FROM THE SEA SQUIRTS *Euherdmania* sp. AND *Eudistoma vannamei* PRODUCE CYTOTOXIC COMPOUNDS

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The sea squirts have been recognized as a rich source of cytotoxic compounds. There are strong evidences pointing the associated microbiota as important producers of cytotoxic compounds isolated from marine invertebrates. Previous study showed highly cytotoxic extracts obtained from the ascidians *Euherdmania* sp and *Eudistoma vannamei* against tumor cell lines [1]. This study aimed to isolate and to evaluate the antitumor potential of microorganisms associated to sea squirts. They were collected at Taiba beach, São Gonçalo do Amarante-CE, Brazil and taken under ice cold refrigeration to the laboratory in Fortaleza-CE. The samples processing was carried out under sterile conditions. Briefly, after cutting a small piece of the colony, they were diluted in sterile sea water followed by heating at 55°C for 10 minutes. The plating was performed with different agar media: sea water agar (SWA), traces metals agar (TMA) and starch casein agar (SCA). The plates were observed until 3 months and individualized colonies were isolated based on actinomycete characteristics. The isolated strains were grown A1 broth medium for seven days. Bacteria samples were cryopreserved in 50% glycerol and frozen at -70°C and included into the bacterial inventory of the laboratory. Isolated strains were grown in 100 mL of A1 (soluble starch, yeast extract and peptone) broth in Erlenmeyer flasks (28°C/200 rpm/10 days), extracted with EtOAc and vacuum-dried. The cytotoxicity was evaluated by the MTT assay against the human colon adenocarcinoma cell line HCT-116 after 72h incubation [2]. Extracts and fractions were tested against HCT-116 cells at concentrations of 5 and 50 µg/ml. The most active extracts were fractionated on a silica column with solvents, and binary mixtures of increasing polarity. 49 strains were isolated from *Euherdmania* sp and 10 from *Eudistoma vannamei*. 67% were recovered from SCA plates. 8 strains (BRA342, BRA345, BRA346, BRA347, BRA401.3, BRA401.4, BRA403, BRA405), yielded cytotoxic extracts, with inhibition concentration mean range from 0.3 to 7 µg/mL. The BRA346E fraction showed inhibition of growth of over 75% in two concentrations, and BRA346F and BRA347B fractions inhibited by 75% at a concentration of 50 µg/mL. Further studies are in progress the isolate the cytotoxic compounds. These results highlight the importance of bioactive compounds of marine origin and reinforces the hypothesis that associated microorganisms produce cytotoxic compounds obtained from marine invertebrates.

References:

- [1] Jimenez, P.C., Fortier, S.C., Lotufo, T.M.C., Pessoa, C., Moraes, M.E.A., Moraes, M.O and Costa-Lotufo, L.V. 2003. Biological activity in extracts of ascidians (Tunicata, Ascidiacea) from the northeastern Brazilian coast. *Journal of Experimental Marine Biology and Ecology*. 287: 93-101.
- [2] Mosmann, T., J. 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Immunol. Methods*, 65: 55-63.