



“FROM THE DEPTHS TO DISCOVERY: CHEMICAL DIVERSITY OF MARINE BACTERIA FROM THE BRAZILIAN CONTINENTAL SHELF”

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Herein, we reveal that deep-sea sediments from the Brazilian continental shelf along the Espírito Santo State coastline, collected in 2015 at approximately 1,500 m depth, harbor a unique diversity of chemically gifted Gram-positive bacteria with biotechnological potential. In this investigation, 41 strains were isolated from wood scraping biofilms and submerged animal bone biofilms preserved in the MicroMarin collection. Recovered strains were subjected to taxonomical identification based on 16S rDNA and their EtOAc extracts were subjected to spectral library annotation by classical molecular networking (CMN) on the GNPS platform, complemented by Dereplicator and Dereplicator+. Among the annotated metabolites, lipopeptides (surfactins, lipopeptide_NO), siderophores (acyl-DFO-D C7–C13), and indole derivatives (tryptamine, N-[2-(1H-indol-3-yl)ethyl]acetamide), are highlighted for their biological associated functions: membrane modulation, iron chelation, and cell signaling. The crude extracts were evaluated in two enzymatic assays (trypsin and chymotrypsin), one antioxidant assay (DPPH), and cytotoxicity assays against HCT-116 and MCF-7 cell lines. Strains identified within the genera *Bacillus*, *Mammaliicoccus*, *Micromonospora*, *Nocardiopsis*, *Streptomyces*, *Verrucosipora*, and *Williamsia* displayed positive activity in both enzymatic and DPPH assays (52.7%), whereas *Bacillus* sp., *Microbacterium* sp., and *Streptomyces* sp. exhibited cytotoxicity (8.1%). *Niallia* sp. BRB-316 strain was selected for further chemical assessments through a fractionation protocol, yielding the S7_3 subfraction obtained by column chromatography guided by TLC, preparative TLC, and purification by microsyringe. ¹H NMR analysis (500 MHz, CDCl₃) revealed key signals consistent with hydroxyl, amine, and methoxy groups. In summary, the integration of CMN, dereplication, and NMR data highlights deep-sea bacteria from the Brazilian continental shelf as a promising chemical reservoir, and the S7_3 subfraction illustrates the potential of rare taxa for biotechnological exploration.

Keywords: Marine Actinomycetota, Protease Inhibition, Cytotoxic Activity, *Niallia* sp., Marine Natural Products

