



Genome mining of *Actinomadura* sp. strain isolated from the São Pedro and São Paulo Archipelago in Brazil

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Abstract: Introduction: Actinomycetes, a phylum of gram-positive mycelial bacteria widely distributed from terrestrial to marine environment, are known as the most prolific producers of pharmacologically active natural products. *Actinomadura* is a genus of this phylum that deserves mention for its capacity of producing cytotoxic natural compounds derived from prodigiosins. The St. Peter and St. Paul Archipelago is a collection of islets located in the equatorial Atlantic Ocean, N00°55.1', W29°20.7', approximately 1010 km to the ENE of the city of Natal, state capital of Rio Grande do Norte (RN) in Brazil. In previous work, our group isolated a marine actinomycete strain, BRA 177, from the sediments of St. Peter and St. Paul Archipelago. In the present work, we evaluate the capacity of BRA 177 to produce bioactive metabolites by characterizing the cytotoxicity and chemical diversity of its crude extract. The 16S rRNA gene and the entire genomic DNA of BRA 177 were also sequenced for molecular identification and prospection of natural products biosynthetic gene clusters respectively. **Methods:** The crude extract of BRA-177 strain showed promising cytotoxic activity through a screening program compared to other 94 isolates. The fermentation broth (20L for 10 days at 150RPM) of the marine *Actinomadura* sp. strain BRA-177 was extracted with ethyl acetate (EtOAc) for one hour under agitation to yield 390,0 mg of crude extract. Chromatographic separations of this led to the isolation of three compounds. Genomic DNA BRA-177 strain was extracted using phenol-chloroform protocol and subjected as template to 16S rRNA PCR amplification, sequencing and phylogeny as well as for shotgun massive NGS at the MiSeq Illumina platform, for exploration of natural products biosynthetic gene clusters. **Results:** BRA-177 ethyl acetate extract showed potent cytotoxic activity against the culture of tumor cell line HCT-116 with IC₅₀ = 4.09µg/mL. The compounds isolated from BRA177 were indicated as nonylprodigiosin, cyclononylprodigiosin and metylcyclooctilprodigiosin. BRA-177 strain was grouped within the genus *Actinomadura* with 57% homology to *Actinomadura madurae*. BRA 177 draft genome prospection led to the detection of 16 ORFs for enzymes with high homology to the ones from *red* and *mar* biosynthetic gene clusters, respectively response for biosynthesis of undecylprodigiosin (and butyl-metacycloheptylprodiginine) and mareniosin in Actinomycetes. The isolation of an *Actinomadura* sp. strain able to produce prodigiosin-family compounds from the pristine environment of St. Peter and St. Paul archipelago reinforces the ecological relevance of these compounds and indicates the potential of such strain as a source of drug leads.