Cytotoxic, mutagenic and antimutagenic screening of *Arenosclera brasiliensis* acetone and ethanol extracts

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Genet. Mol. Res. 7 (2): 542-548 (2008)
Received March 18, 2008
Accepted June 3, 2008
Published June 17, 2008

ABSTRACT. The marine environment is a rich source of biologically active compounds with pharmacological properties. Marine organisms often produce secondary metabolites with structural features different from those produced by terrestrial ones, and the Phylum Porifera seems to be one of the most productive in this sense. This study was undertaken to provide data on mutagenic and antimutagenic activities from an acetone (Areac) and an ethanol (Areet) extract obtained from *Arenosclera brasiliensis*, an endemic Brazilian sponge. A qualitative *Salmonella* reverse mutation test was performed with the TA97, TA98, TA100, and TA102 strains by incubating cells

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with Areac and Areet in the presence and absence of a known mutagen. A cytotoxic evaluation of the extracts was also performed. *A. brasiliensis* did not display any mutagenic activity, but Areac showed significant toxicity against test strains. In the antimutagenic assay, a reduction in the number of *his*$^+$ revertants was observed for the TA97, TA100 and TA102 strains treated with Areac when compared to the positive controls. Areet treatment showed protective activity against DNA lesions only for the TA100. These results are in agreement with those obtained previously with other *A. brasiliensis* extracts, suggesting an antimutagenic activity.

Key words: *Arenosclera brasiliensis*; Antimutagenicity; *Salmonella* reverse mutation test; Marine natural products

INTRODUCTION

Nature is an attractive source of new therapeutic compounds with a tremendous diversity found among millions of species of plants, animals, marine organisms, and microorganisms (da Rocha et al., 2001). The marine environment is a promising source of biologically active compounds, especially in Brazil where the coastline extends about 8000 km and comprises over 800,000 km^2 of continental shelf still poorly explored. The marine fauna and flora usually produce secondary metabolites with structural features distinct from other natural sources which are of interest for potential industrial and medical applications (Berlinck et al., 2004; Hausman et al., 2006; Aiub et al., 2006).

The marine sponges (Phylum Porifera) have the greatest diversity of active secondary metabolites (Van Soest and Braekman, 1999). Most of them have unique chemical patterns and generally have anti-inflammatory, antitumor, antibiotic, antiviral, or antifouling activities (Sipkema et al., 2005).

Our source of study was the marine sponge *Arenosclera brasiliensis* (Demospongiae, Haplosclerida) which is endemic to the southwest coast of Brazil (Torres et al., 2000). The order Haplosclerida is an important source of alkylpiperidine alkaloids, with a great diversity of compounds reported (Almeida and Berlinck, 1997).

The present study was undertaken to screen for chemical groups of active components in acetone and ethanol extracts of *A. brasiliensis* and to provide data on the genotoxic and antigenotoxic activity present.

MATERIAL AND METHODS

Preparation and infrared and ultraviolet analysis of *A. brasiliensis* extracts

Samples of *A. brasiliensis* were collected at João Fernandinho Beach, Búzios (Rio de Janeiro, RJ, Brazil), in April 2006 at a depth of 2-7 meters. Samples were washed with sea water, cleaned of all visible surface debris, and stored at 4°C for 6 months, in 92% ethanol in a volume corresponding to 2-fold its weight. A voucher sample was deposited in the Porifera collection of Museu Nacional do Rio de Janeiro (MNRJ 1704).

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To prepare the acetone (Areac) and ethanol (Areet) extracts of *A. brasiliensis*, 106 g tissue sample was submitted to an extraction using a Soxhlet extractor with 300 mL acetone (99.5%) and ethanol (96%) as solvents. After the first cycle, samples were submitted to extraction for two more hours. Acetone and ethanol were evaporated under vacuum at 45° and 55°C and the extracts were lyophilized, yielding 143.7 and 61.9 dry weight, respectively. Both extracts were frozen at -20°C and diluted in distilled water before use.

The chemical analysis of the extracts consisted of measuring the ultraviolet spectra of the final fractions (diluted in distilled water and ethanol, 1:100), recorded with a Shimadzu UV-160A spectrophotometer, and the infrared spectra (FTIR), recorded with a Perkin-Elmer Spectrum One instrument with a resolution of 4 cm^{-1} with the dried extracts dissolved in chloroform and deposited in a liquid cell with a KBr window.

Bacterial strains

The *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 were provided by Dr. B.N. Ames, University of California, Berkeley, CA, USA.

Qualitative *Salmonella* reverse mutation assay

Mutagenicity and antimutagenicity were evaluated in both Areac and Areet, following the protocol proposed by Maron and Ames (1983), with some adaptations. A 100-μL aliquot of an overnight cell culture (2 x 10^9 cells/mL) of *S. typhimurium* strains TA97, TA98, TA100, and TA102 was plated with 2 mL of top agar (0.7% agar, 0.6% NaCl, 50 μM L-histidine, 50 μM biotin, pH 7.4, 40°C) on Petri dishes containing 1.5% agar in Vogel Bonner E medium with 2% glucose (minimal agar medium). After solidification of the top agar, filter disks of approximately 5 mm were placed on the middle of all Petri dishes.

For the mutagenic test, 10 μL Areac (1.2 μg/plate) and 10 μL Areet (2.3 μg/plate) were poured on the filter disks, and for the antimutagenic test, 10 μL Areac and Areet (1.2 and 2.3 μg/plate, respectively) together with 10 μL of the respective positive control were poured on the filter disks. Afterward, the strains were incubated at 37°C for 72 h, *his*$^+$ revertants were counted, and if an inhibition halo formed, it was measured. Positive controls used in the assay were 4-nitroquinoline 1-oxide (0.5 ng/plate) for the TA97 strain, 2-aminofluorene (0.015 ng/plate) for the TA98 strain, sodium azide (50 ng/plate) for the TA100 strain, and mitomycin C (2.5 ng/plate) for the TA102 strain. The tests were performed in triplicate and repeated twice.

Cytotoxicity

For Areac and Areet cytotoxic determination, 10 μL of each (1.2 and 2.3 μg/plate, respectively) were poured on the filter disks placed on the middle of Petri dishes, containing nutrient agar medium (0.8% Bacto nutrient broth (Difco), 0.5% NaCl and 1.5% agar). The plates were incubated at 37°C for 24 h and the inhibition halo, when present, was measured. Experiments were performed in triplicate and repeated twice.

RESULTS AND DISCUSSION

The FTIR of the extracts showed the following absorption peaks: 3411, 2924, 2853, 1634, 1405, 1195, 1044, and 742 cm^{-1} for Areac and 3412, 1634, 1404, 1199, and 1044 cm^{-1} for Areet. The spectra obtained by ultraviolet (UV) analysis corroborate the functional groups detected in Areet and Areac extracts by FTIR.

The chemical groups detected are also the same described for arenosclerins A-C and haliclonacyclamine E, previously isolated compounds from *A. brasiliensis* (Torres et al., 2000), and for Arecr and Arefil, *A. brasiliensis* extracts that have shown antimutagenic activity (Stankevicins L, Aiub C, de Santa Maria LC, Lobo-Hajdu G, and Felzenszwalb I, unpublished results).

No mutagenicity was observed in the *S. typhimurium* qualitative reverse mutation assay for Areac. The TA97, TA100 and TA102 strains showed a reduction in the number of revertants in the antimutagenic assay performed with Areac, when compared to the positive controls (Figures 1-4). No mutagenic activity was detected in strains treated with Areet. Antimutagenicity was detected only for the TA100 strain.

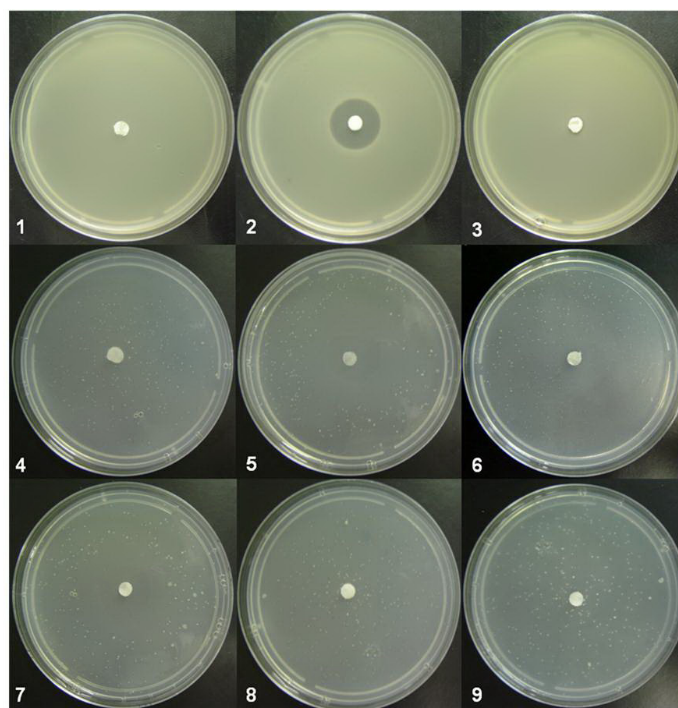

Figure 1. Experiments performed with the TA97 strain. Survival experiment of **1.** Negative control (0.9% NaCl), **2.** Areac at 1.2 µg/plate and **3.** Areet at 2.3 µg/plate. *Salmonella* reverse mutation assay of **4.** Negative control (0.9% NaCl), **5.** Areac at 1.2 µg/plate and **6.** Areet at 2.3 µg/plate. Antimutagenic analysis of **7.** Areac at 1.2 µg/plate, **8.** Areet at 2.3 µg/plate and **9.** Positive control described in Material and Methods.

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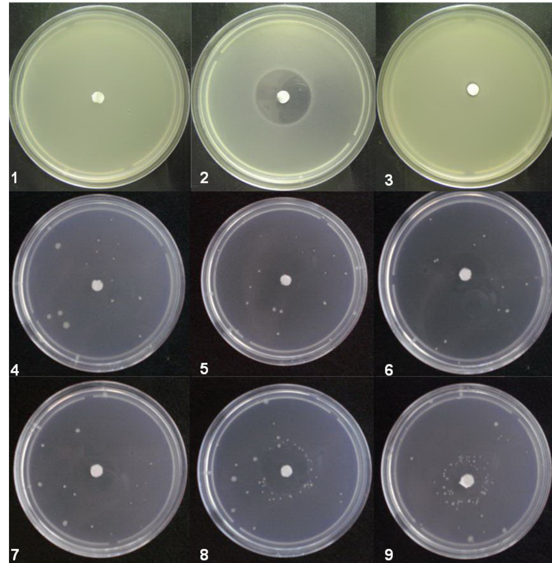

Figure 2. Experiments performed with the TA98 strain. Survival experiment of **1.** Negative control (0.9% NaCl), **2.** Areac at 1.2 μg/plate and **3.** Areet at 2.3 μg/plate. *Salmonella* reverse mutation assay of **4.** Negative control (0.9% NaCl), **5.** Areac at 1.2 μg/plate and **6.** Areet at 2.3 μg/plate. Antimutagenic analysis of **7.** Areac at 1.2 μg/plate, **8.** Areet at 2.3 μg/plate and **9.** Positive control described in Material and Methods.

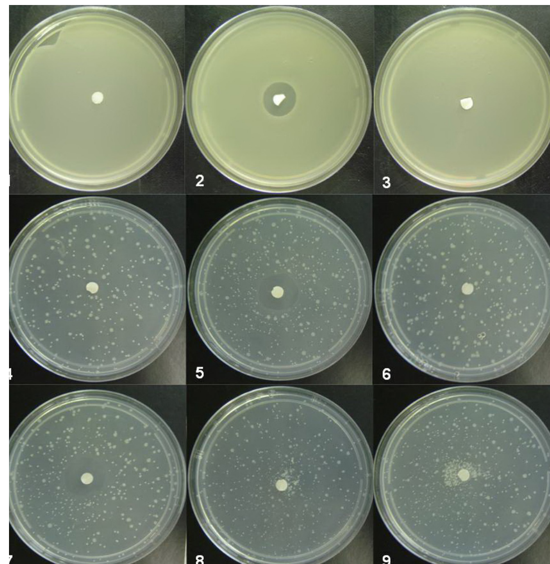

Figure 3. Experiments performed with the TA100 strain. Survival experiment of **1.** Negative control (0.9% NaCl), **2.** Areac at 1.2 μg/plate and **3.** Areet at 2.3 μg/plate. *Salmonella* reverse mutation assay of **4.** Negative control (0.9% NaCl), **5.** Areac at 1.2 μg/plate and **6.** Areet at 2.3 μg/plate. Antimutagenic analysis of **7.** Areac at 1.2 μg/plate, **8.** Areet at 2.3 μg/plate and **9.** Positive control described in Material and Methods.

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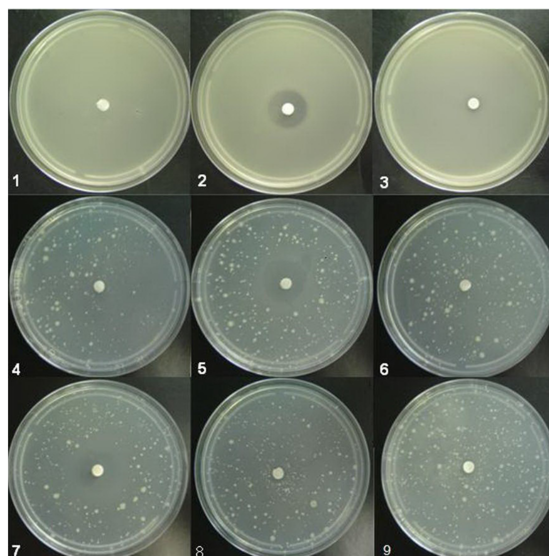

Figure 4. Experiments performed with the TA102 strain. Survival experiment of **1.** Negative control (0.9% NaCl), **2.** Areac at 1.2 μg/plate and **3.** Areet at 2.3 μg/plate. *Salmonella* reverse mutation assay of **4.** Negative control (0.9% NaCl), **5.** Areac at 1.2 μg/plate and **6.** Areet at 2.3 μg/plate. Antimutagenic analysis of **7.** Areac at 1.2 μg/plate, **8.** Areet at 2.3 μg/plate and **9.** Positive control described in Material and Methods.

In survival assays, an inhibition halo of approximately 18 mm for TA97, 26 mm for TA98, 14 mm for TA100, and 17 mm for TA102 could be observed when treated with Areac. No cytotoxic effect was observed for Areet when compared to the negative control, treated with 0.9% NaCl.

These results are in agreement with those obtained previously with Arefil extract (Stankevicins L, Aiub C, de Santa Maria LC, Lobo-Hajdu G, and Felzenszwalb I, unpublished results), suggesting an important protective effect against lesions caused by different classes of mutagens.

ACKNOWLEDGMENTS

The authors are thankful to Rita Maria P. de Sá, Departamento de Química Orgânica, UERJ, Brazil, for the spectrometric analysis. Research supported by FAPERJ, CNPq, SR-2-UERJ, and CAPES.

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