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Antibiotic resistance genes detected in the marine sponge *Petromica citrina* from Brazilian coast

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ABSTRACT

Although antibiotic-resistant pathogens pose a significant threat to human health, the environmental reservoirs of the resistance determinants are still poorly understood. This study reports the detection of resistance genes (*ermB*, *mecA*, *mupA*, *qnrA*, *qnrB* and *tetL*) to antibiotics among certain culturable and unculturable bacteria associated with the marine sponge *Petromica citrina*. The antimicrobial activities elicited by *P. citrina* and its associated bacteria are also described. The results indicate that the marine environment could play an important role in the development of antibiotic resistance and the dissemination of resistance genes among bacteria.

Introduction

The spread of antibiotic-resistant microorganisms in the environment is globally recognized as an important public health issue, and there are concerns on our future ability to treat infectious diseases.[1] Therefore, the knowledge of the nature of these resistance determinants in natural habitats is indispensable to get a better insight of the development of antibiotic resistance in clinical settings.[2]

In a previous publication, Marinho and colleagues[3] demonstrated the antimicrobial and cytotoxic activities of the compound halistanol trisulphate isolated from *P. citrina*. This compound exhibited a broad-spectrum antibacterial activity against certain medically important bacteria, including resistant strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*,

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Enterococcus faecalis, *Mycobacterium fortuitum* and *Neisseria gonorrhoeae*.[3]

Symbiotic microbial communities can significantly impact the host-sponge ecology and evolution through supplemental nutrition and by the production of bioactive substances that can deter predators, competitors, and fouling organisms. Many of these substances possess antibacterial activity.[4] The microbes that produce these antibiotics harbor resistance genes to protect themselves. Therefore, the selective pressure of the environment shapes these bacterial communities.[5]

In this background, the aim of the present study was to detect the resistance genes in culturable and unculturable bacteria associated with the sponge *P. citrina*. This study is the first report detecting the antibiotic resistance genes in *P. citrina* by culture-independent approaches. Such genes have usually been described in pathogenic bacteria.

Material and methods

Sponge collection and bacteria used in this study

The samples of the sponge *P. citrina* were collected by scuba-diving at a depth of 4–20 m at Cagarras Archipelago (23801′S–43811′W), located in Rio de Janeiro, south-eastern Brazil (south-western Atlantic).

The bacterial strains were isolated and identified from *P. citrina* by Santos-Gandelman and colleagues in an earlier study.[6] Of them, six were selected according to their antibacterial activity against certain medically important strains[7] and/or antibiotic resistance profile.[6] *Bacillus* Pc31 and Pc32, *Enterococcus* Pc5b and *Shigella* Pc5a strains were grown in brain–heart infusion medium (BHI) (Difco, MI, USA), and *Bacillus* Pc3M and *Halomonas* Pc51M were grown in a marine medium (Marine 2216, Difco), at 25 °C for 24 h.

The following strains were included as positive controls for specific amplification of the different genes under investigation: *Escherichia coli* LO (*qnrA*), *E. coli* EB2b (*qnrB*), *Streptococcus agalactiae* (*ermB*), *S. agalactiae* CL5596 (*tetL*), *Staphylococcus haemolyticus* MD2 (*mecA* and *mupA*). These strains were grown in BHI medium at 37 °C for 18 h.

Polymerase chain reaction amplification

DNA from 0.25 g of the sponge body was extracted using the Ultra Clean Soil DNA isolation kit (Mo Bio, Carlsbad, CA, USA) following the manufacturer's protocol. DNA from the bacterial strains was isolated by the guanidinium thiocyanate extraction method.[8]

Thus, the total DNA isolated from the bacteria from the sponge samples and from the culturable bacteria isolated from *P. citrina* were used to amplify genes conferring resistance to macrolide-lincosamide-streptogramin (*ermB*), methicillin (*mecA*), mupirocin (*mupA*), quinolones (*qnrA*, *qnrB*), and tetracyclines (*tetL*).

The following primers were used: for *ermB*, F: 5-CATTTAACGACGAAACTGGC and R: 5-GGAACATCTGTGGTATGGCG,[9] to give a 425-bp product; for *mecA*, F: 5-TAGAAATGACTGAACGTCCG and R: 5-TTGCGATCAATGTTACCTAG,[10] to give a 154-bp product; for *mupA* F: 5-GTTTATCTTCTGATGCTGAG and R: 5-CCCCAGTTACACCGATATAA,[11] to give a 237-bp product; for *qnrA*, F: 5-ATTTCTCACGCCAGGATTTG and R: 5-GATCGGCAAAGGTTAGGTCA,[12] to give a 516-bp product; for *qnrB*, F: 5-GATCGTGAAAGCCAGAAAGG and R: 5-ACGATGCCTGGTAGTTGTCC,[12] to give a 469-bp product; for *tetL*, F: 5-ATAAATTGTTTCGGGTCGGTAAT and R: 5-AACCAGCCAACTAATGACAATGAT,[13] to give a 1077-bp product.

The reaction mixtures, in final volumes of 50 μL, contained $MgCl_2$ (1.5 mM for the *mecA* and *mupA* genes; 2 mM for the *ermB* and *tetL* genes, and 4 mM for the *qnrA* and *qnrB* genes), deoxynucleoside triphosphates (0.2 mM each), primers (0.5 μM each), *Taq* DNA polymerase (0.5 U), reaction buffer (10 mM), and 10–20 ng of the extracted DNA as the template.

The PCR conditions were initial denaturation at 94 °C for 5 min, followed by 32 cycles at 94 °C for 45 s, 53 °C for 45 s, and 72 °C for 60 s, with a final elongation step at 72 °C for 5 min.[12] The positive (strains with known resistance genes) and negative (without DNA template) controls were included in each run. Amplification products were provisionally identified from their sizes in ethidium bromide-stained agarose gels.

Results and discussion

The information about the selection pressures on antibiotic resistance genes is very limited regarding the remote environments with low direct human contacts. A more comprehensive understanding of the natural roles of putative antibiotic resistance genes is crucial in understanding of their origin and functions.[14]

In recent years, several antibiotics and other bioactive molecules have been isolated from marine sponges[15] and from sponge-associated bacteria,[4,16] including *P. citrina*[3] and its associated bacteria.[7]

The *P. citrina* samples were collected at Cagarras Archipelago, which is a recent marine protected area located on the coast of Rio de Janeiro, Brazil. These islands are impacted both by the Guanabara Bay waters and by the discharges from a submarine outfall that releases untreated domestic sewage, both of which are balanced by the influx of pristine offshore water masses.[17]

In this study, resistance genes for different antibiotics were detected in the DNA extracted from the culturable and unculturable bacteria associated with the sponge *P. citrina*. All amplicons were of the sizes of those of the positive controls (Table 1). The antibiotic resistance profile of the culturable bacteria associated with *P. citrina* has already been reported.[6] This conforms to the data reported herein, as we have reported genes for quinolone and erythromycin resistance. Besides, the results also indicate that the hologenome of *P. citrina* contains genes encoding antibiotic resistance to erythromycin, methicillin, mupirocin, quinolone, and tetracycline. This goes in line with the fact that many marine sponges harbor dense and diverse microbial communities of considerable ecological and biotechnological importance.[5]

The application of culture-independent approaches, such as PCR and metagenomics, for the study of antibiotic resistance genes in the environment has uncovered a vast diversity of antibiotic resistance genes in soil bacteria. However, according to the best of our knowledge, this is the first time that