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Antagonistic Interactions among Bacteria Isolated from either the Same or from Different Sponges Native to the Brazilian Coast

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Abstract

Marine sponges are sessile and filter-feeding organisms that harbor dense and diverse microbial communities of considerable ecological and biotechnological importance. They represent an important target for the study of bacterial interactions in marine ecosystems. The purpose of this study was to examine the frequency of antagonistic interactions among the culturable microbial communities associated with sponges from the Brazilian coast. The specimens were collected over six years at Cagarras Archipelago, Praia Vermelha Beach and Urca square, Rio de Janeiro State, SE Brazil. Fifty-six bacterial isolates representing four classes of cultivable sponge-associated bacteria were studied for their ability to produce inhibitory substances. Antagonistic interactions occurred among isolates from both, the same and different sponge species. Most isolates (98.2%) were able to inhibit growth of at least one indicator bacterium. In contrast, there were few antagonistic interactions among bacteria obtained from the same sponge specimen. Our results suggest that chemical antagonism could play a significant role in shaping the bacterial communities within sponge tissues.

Keywords: Antagonism; Cultivable bacteria; Inhibitory substances; Sponge microbial communities

Introduction

Associations between sponges and bacteria have existed for over 600 million years and are one of the most ancient of all symbioses between microbes and metazoa [1]. Most sponges host diverse and abundant communities of microorganisms, which contribute to host health, ecology and evolution [2,3]. The relationship between sponges and their associated microbial communities is so important that microorganisms can contribute to more than 35% of the sponge biomass [4] and may undertake diverse functional roles including nutrition, cycling of metabolites and host defense [5].

The phylum Porifera includes approximately 8,700 valid species known worldwide [6]. Of these, 515 species have so far been identified in Brazil [7]. There is a lack of reliable baseline data on the composition and stability of symbiotic microbial communities for most sponge species. This knowledge gap makes it difficult to determine the role of microorganisms in health, diseases and mortality of sponges. This role has been studied for coral-associated bacteria [8], where inhibitory activities measured towards known coral pathogens have led to the hypothesis that bacteria associated with healthy corals play a protective role for the coral holobionts [9]. An ecological role of antagonism has also been suggested for bacteria associated with Antarctic sponges [10], brittle stars [11], and marine aggregates [12].

In this study, we tested the hypothesis that chemical antagonism is common among cultivable bacteria associated with sponges. For this purpose, a total of 27 sponge samples representing 13 species were collected (between 2005 and 2011) at the coast of Rio de Janeiro city, Brazil. We measured the frequency of antagonistic interactions among the culturable microbial communities associated with either the same or different sponge species from the same area. More specifically, we aimed to address the following questions: How frequent is antagonism between sponge-associated bacteria? Is this frequency modulated by taxonomic identity, sponge specimen, sampling site and/or sampling date of the sponge?

Material and Methods

Sponge collection and bacterial isolation and cultivation

A total of 27 sponge samples representing 13 sponge species were collected between 2005 and 2011 by scuba diving at depths of 4-20 m, at 18–25°C in the Cagarras Archipelago (CA) (23°01'S, 43°11'W), Praia Vermelha beach (PV) (22°57'S, 43°09'W) and Urca square (Us) (22°95'S, 43°16'W), located at the coast of Rio de Janeiro, Brazil (Table 1) [13].

These three different sites (CA, PV and Us) are located within a perimeter of about 10 km. QU and PV are dynamic ecosystems located at the interface between the Guanabara Bay eutrophic (polluted) estuarine waters and the adjacent coastal Atlantic Ocean seawaters. CA is located further away from the bay and is under the influence of the Brazil Current and the South Atlantic Central waters [14].

Specimens were macerated at room temperature (RT, 25 ± 2°C) in

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Sponge species	Voucher MMBL[a]	Colletion site[b]	Marine strains[c]	Bacterial genera	Phylum/Class
Clathrina aurea	42006Ca	PV	Ca31	*Pseudovibrio* sp.	Proteobacteria /Alphaproteobacteria
Cliona aff. celata	92010Cc	Us	Cc81	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Cliona aff. celata	92010Cc	Us	Cc82	*Citrobacter* sp.	Proteobacteria /Gammaproteobacteria
Cliona aff. celata	82011Cc	Us	Cc92	*Citrobacter* sp.	Proteobacteria /Gammaproteobacteria
Cliona aff. celata	82011Cc	Us	Cc93	*Rhodococcus* sp.	Actinobacteria
Cliona aff. celata	82011Cc	Us	Cc94	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Dragmacidon reticulatum	42006Dr	PV	Dr32	*Lactococcus* sp.	Firmicutes / Bacilli
Dragmacidon reticulatum	42006Dr	PV	Dr34	*Psychrobacter* sp.	Proteobacteria /Gammaproteobacteria
Dragmacidon reticulatum	42006Dr	PV	Dr35	*Psychrobacter* sp.	Proteobacteria /Gammaproteobacteria
Dragmacidon reticulatum	42006Dr	PV	Dr36	*Brachybacterium* sp.	Actinobacteria
Dragmacidon reticulatum	42006Dr	PV	Dr37	*Brachybacterium* sp.	Actinobacteria
Dragmacidon reticulatum	32007Dr	PV	Dr5	*Enterococcus* sp.	Firmicutes / Bacilli
Dragmacidon reticulatum	62009Dr	CA	Dr72	*Kokuria* sp.	Actinobacteria
Geodia corticostylifera	32007Gc	CA	Gc51	*Serratia* sp.	Proteobacteria /Gammaproteobacteria
Geodia corticostylifera	32007Gc	CA	Gc54	*Lactococcus* sp.	Firmicutes / Bacilli
Haliclona vansoesti	12007Hv	CA	Hv40	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Haliclona vansoesti	12007Hv	CA	Hv41	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Haliclona fugidia	32007Hf	PV	Hf51	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Haliclona fugidia	32007Hf	PV	Hf52	*Enterococcus* sp.	Firmicutes / Bacilli
Hymeniacidon heliophila	32007Hh	PV	Hh5	*Acinetobacter* sp.	Proteobacteria /Gammaproteobacteria
Hymeniacidon heliophila	92010Hh	Us	Hh81	*Bacillus* sp.	Firmicutes / Bacilli
Hymeniacidon heliophila	92010Hh	Us	Hh82	*Bacillus* sp.	Firmicutes / Bacilli
Hymeniacidon heliophila	82011Hh	Us	Hh91	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Hymeniacidon heliophila	82011Hh	Us	Hh92	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Hymeniacidon heliophila	82011Hh	Us	Hh93	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Hymeniacidon heliophila	82011Hh	Us	Hh94	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	52005Mm	PV	Mm1	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	52005Mm	PV	Mm3	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Mycale microsigmatosa	42006Mm	PV	Mm31	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	42006Mm	PV	Mm32	*Shigella* sp.	Proteobacteria /Gammaproteobacteria
Mycale microsigmatosa	42006Mm	PV	Mm33	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	42006Mm	PV	Mm35	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	32007Mm	CA	Mm51a	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	32007Mm	CA	Mm51b	*Lactococcus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	92010Mm	Us	Mm81	*Pseudomonas* sp.	Proteobacteria /Gammaproteobacteria
Mycale microsigmatosa	92010Mm	Us	Mm82	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	92010Mm	Us	Mm84	*Bacillus* sp.	Firmicutes / Bacilli
Mycale microsigmatosa	82011Mm	Us	Mm91	*Enterococcus* sp.	Firmicutes / Bacilli
Pachychalina alcaloidifera	32007Pa	CA	Pa51	*Bacillus* sp.	Firmicutes / Bacilli
Pachychalina alcaloidifera	32007Pa	CA	Pa52	*Enterococcus* sp.	Firmicutes / Bacilli
Pachychalina alcaloidifera	32007Pa	CA	Pa53	*Bacillus* sp.	Firmicutes / Bacilli
Petromica citrina	42006Pc	CA	Pc31	*Bacillus* sp.	Firmicutes / Bacilli
Petromica citrina	42006Pc	CA	Pc32	*Bacillus* sp.	Firmicutes / Bacilli
Petromica citrina	32007Pc	CA	Pc5a	*Shigella* sp.	Proteobacteria /Gammaproteobacteria
Petromica citrina	32007Pc	CA	Pc5b	*Enterococcus* sp.	Firmicutes / Bacilli
Polymastia janeirensis	52005Pj	PV	Pj1	*Bacillus* sp.	Firmicutes / Bacilli
Polymastia janeirensis	52005Pj	PV	Pj2	*Bacillus* sp.	Firmicutes / Bacilli
Polymastia janeirensis	42006Pj	PV	Pj32	*Lactococcus* sp.	Firmicutes / Bacilli
Polymastia janeirensis	42006Pj	PV	Pj33	*Klebsiella* sp.	Proteobacteria /Gammaproteobacteria
Polymastia janeirensis	32007Pj	PV	Pj52	*Lysinibacillus* sp.	Firmicutes / Bacilli
Paraleucilla magna	12007Pm	CA	Pm42	*Citrobacter* sp.	Proteobacteria /Gammaproteobacteria
Paraleucilla magna	32007Pm	CA	Pm52	*Bacillus* sp.	Firmicutes / Bacilli

Tedania ignis	12007Ti	CA	Ti41	*Brevibacillus* sp.	Firmicutes / Bacilli
Tedania ignis	32007Ti	CA	Ti54	*Staphylococcus* sp.	Firmicutes / Bacilli
Tedania ignis	32007Ti	CA	Ti55	*Citrobacter* sp.	Proteobacteria /Gammaproteobacteria
Tedania ignis	32007Ti	CA	Ti56	*Citrobacter* sp.	Proteobacteria /Gammaproteobacteria

[a]Voucher number of the marine sponges collection of the Molecular and Marine Bacteriology Laboratory (MMBL) of the Microbiology Institute, UFRJ, Brazil.
[b]All the specimens of sponges were collected by scuba diving at depths of 4-2 m, at 18-25°C, in the Cagarras Archipelago (CA), Praia Vermelha beach (PV) and Urca square (Us), Rio de Janeiro, Brazil.
[c]The strains belong to the sponge-associated bacteria collection of the Molecular and Marine Bacteriology Laboratory (MMBL) of the Microbiology Institute, UFRJ, Brazil.

Table 1: Sponges, collection sites and isolated bacteria.

brain-heart infusion medium (BHI) (Difco). Subsequently, macerates were serially 10-fold diluted, inoculated in replicates on BHI agar and incubated for up to seven days at RT. Bacteria were purified from the primary culture and kept in slant cultures at -20°C [15].

16S rRNA sequence analysis of bacterial isolates

Bacterial DNA was recovered by a thermal lysis protocol consisting in re-suspending cellular material from each colony in 25 µl sterile PCR grade water and boiling the suspension at 100°C for 15 min. PCR amplification was performed by adding 3 µl DNA solution to 47 µl containing 1× buffer GO TAQ green master mix (Promega), BSA 0.4 mg/ml, Igepal 0.05%, and 20 pmol of each universal primer, 27F (5′-GAGTTTGATCMTGGCTCAG- 3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) [16], Cycle conditions consisted in an initial denaturation step at 94°C for 6 min, followed by 30 cycles at 94°C for 30 s, 55°C for 1 min 30 s and 72°C for 2 min 30 s, and a final elongation step at 72°C for 5 min.

PCR products were confirmed by electrophoresis on a 0.8% agarose gel, purified using the QIAquick PCR Purification Kit (Qiagen), and sequenced using the universal primer 338F (5′-ACTCCTACGGGAGGCAGC-3′) at Beckman Coulter Genomics (Takeley, UK). 16S rRNA gene sequences obtained for the isolates were aligned and classified using the online portal of the SILVA SINA alignment service of the ARB-Silva database (http://www.arb-silva.de/aligner/) [17].

Antagonistic interactions among bacterial isolates

Bacterial strains were screened for antagonistic interactions by a previously described method to evaluate production of antimicrobial substances [18]. Hereafter, bacterial strains tested for antimicrobial substance production will be termed "producer" strains, whereas those used as targets will be called "indicator" strains. Briefly, 10^7 cells of each producer strain were spotted onto BHI-agar and incubated at 25°C until the colony diameter reached 8 mm. In parallel, each indicator strain was grown in liquid medium at 25°C for 24-48 h. Subsequently, 10^5 cells of the indicator culture were mixed with 3 ml of BHI soft agar and poured over the plates. Plates were incubated at 25°C for 24 h and the diameter of the inhibition zone around the spotted strain was measured. An indicator strain was considered sensitive to the activity of the producer strain when it exhibited a clear inhibition zone with a diameter ≥ 8 mm. When the inhibition zone was <8 mm or when bacterial colonies grew inside the inhibition zone, the indicator strain was considered resistant [18].

A total of 3,080 tests, i.e. a 56 × 55 array, were performed for assessing antagonistic interactions among the bacterial strains. This means that each strain was tested against the 55 other strains for cross-inhibition. In order to calculate the relative frequencies of antagonistic interactions, the number of antagonistic interactions observed for a given strain (absolute frequency) was divided by the total number of interactions performed with this strain.

Bacterial isolates were then operationally grouped into three different interactivity clusters: (I) active, if they were able to inhibit the growth of at least one indicator strain; (II) sensitive, if their growth was inhibited by at least one producer strain; and (III) resistant, if their growth was not inhibited by any producer strain. It must be noted that an individual strain could be simultaneously included in the interactivity clusters I and II (active and sensitive), or I and III (active and resistant), but never in II and III [10].

The antagonistic relationships were also plotted in network graphs using the program Cytoscape 3.1.0 (http://www.cytoscape.org).

Results

Phylogenetic affiliation of isolates

Fifty-six bacterial strains were isolated from the 13 sampled sponge species. Based on 16S rRNA gene sequence analysis (targeting the V3-V5 region), they represented four classes: Firmicutes (52%), Gammaproteobacteria (39%), Actinobacteria (7%) and Alphaproteobacteria (2%). The most frequently isolated genus was *Bacillus* (17 isolates), followed by *Pseudomonas* (10 isolates), *Citrobacter* (5 isolates), *Enterococcus* (5 isolates) and *Lactococcus* (4 isolates). Other genera isolated in smaller numbers included *Brachybacterium*, *Psychrobacter* and *Shigella* (2 isolates each), and *Acinetobacter*, *Brevibacillus*, *Klebsiella*, *Kocuria*, *Lysinibacillus*, *Pseudovibrio*, *Rhodococcus*, *Serratia* and *Staphylococcus* (1 isolate each) (Table 1).

Antagonistic interactions among bacterial isolates

The 56 isolated bacterial strains were screened against each other in 3,080 cross-tests (56 × 55 tests) for antagonistic interactions by a protocol to test for antimicrobial substance production (Table 2).

General cross-inhibition among bacteria isolated from different species of sponges collected of the different sites between 2005 and 2011

General cross-inhibition among bacteria isolated from all sponge samples showed a relative frequency of 18% (555 positive tests out of 3,080). The diameter of the inhibition zone varied from 10 to 38 mm (Table 2 and Figure 1). In total, 55 of out 56 isolates (98.2%) were active, i.e. producing antimicrobial substances against at least one indicator strain (Figure 2a). In these cross-inhibition tests, several active strains also proved to be sensitive to the effects of other tested isolates (Figure 2b). Actually, all isolates were sensitive, as their growth was inhibited by at least one strain used as a producer.

Each producer strains inhibited a mean of 10 indicator strains. While the majority of producers inhibited between 6 and 12 indicator strains, eight producer strains inhibited 15 or more indicator strains (Figure 3).

Bacteria belonging to all 17 cultured genera showed inhibitory

Indicator[a]	Pa51	Pa52	Pa53	Ca31	Cc81	Cc82	Cc92	Cc93	Cc94	Dr32	Dr34	Dr35	Dr36	Dr37	Dr5	Dr72
Producer[a]																
Pa51		0	0	0	0	0	10[b]	10	0	0	0	0	0	0	0	0
Pa52	0		0	0	0	10	10	10	0	0	0	0	0	0	0	0
Pa53	0	0		0	0	0	0	10	0	0	0	0	0	0	0	0
Ca31	0	0	0		0	0	10	10	0	0	0	0	0	0	0	0
Cc81	10	0	0	0		0	0	0	0	0	0	0	0	0	0	0
Cc82	10	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Cc92	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0
Cc93	0	0	0	0	0	0	0		0	0	0	0	0	10	10	10
Cc94	25	23	28	0	0	0	0	0		26	24	0	26	22	25	22
Dr32	0	0	0	0	0	0	0	10	0		0	0	0	0	0	0
Dr34	0	0	0	0	0	0	0	10	0	0		0	0	0	0	0
Dr35	0	0	0	0	0	0	0	15	0	0	0		0	0	0	0
Dr36	0	0	0	0	0	0	10	10	0	0	0	0		0	0	0
Dr37	0	0	0	0	10	0	10	0	0	0	0	0	0		10	0
Dr5	0	0	0	0	0	0	10	0	0	0	0	0	0	0		0
Dr72	0	0	0	0	0	0	10	0	0	0	0	0	0	0	10	
G51	0	0	0	0	10	10	10	24	0	0	36	32	0	0	10	0
G54	0	0	0	0	0	0	0	10	0	0	0	10	0	0	10	0
Hv40	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hv41	18	22	25	22	0	0	0	10	0	22	25	28	28	22	22	35
Hf51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hf52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hh5	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0
Hh81	25	0	25	20	10	0	0	0	0	24	20	0	0	0	0	0
Hh82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hh91	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0
Hh92	0	0	0	0	0	0	22	0	0	0	10	10	0	0	0	0
Hh93	30	35	33	30	0	0	20	0	0	25	42	15	34	0	10	0
Hh94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm3	0	0	0	0	0	0	20	10	0	0	0	0	14	0	0	0
Mm31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm32	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm51a	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
Mm51b	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
Mm81	12	0	13	12	0	0	0	0	0	12	16	12	0	0	0	0
Mm82	13	0	13	12	0	0	0	0	0	12	16	12	0	0	0	0
Mm84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm91	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
Pc31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pc32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pc5a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pc5b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pj1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pj2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pj32	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0
Pj33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pj52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pm42	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0
Pm52	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0
Ti41	0	10	0	0	0	0	0	0	0	0	10	10	0	0	0	0

Ti54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ti55	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0
Ti56	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0

Indicator	G51	G54	Hv40	Hv41	Hf51	Hf52	Hh5	Hh81	Hh82	Hh91	Hh92	Hh93	Hh94	Mm1	Mm3
Producer															
Pa51	0	0	0	0	0	10	0	0	0	0	10	0	0	0	0
Pa52	0	10	0	0	0	10	0	0	0	0	10	0	0	0	0
Pa53	0	38	0	0	0	10	0	0	0	0	10	0	0	0	0
Ca31	0	0	0	0	0	10	0	0	0	0	10	0	0	0	0
Cc81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cc82	0	10	0	0	0	0	0	0	0	0	0	0	0	10	0
Cc92	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cc93	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
Cc94	10	0	0	25	0	26	28	0	0	18	0	0	15	18	0
Dr32	0	0	0	0	0	10	0	0	0	0	10	0	0	0	0
Dr34	0	10	0	0	0	0	0	0	0	0	10	0	0	0	0
Dr35	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0
Dr36	0	10	0	0	0	10	0	0	0	0	10	0	0	0	0
Dr37	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0
Dr5	0	10	0	0	0	10	0	0	0	0	10	0	0	0	0
Dr72	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
G51		12	12	0	0	14	0	0	0	0	0	0	10	14	0
G54	0		0	0	0	0	0	10	10	0	0	0	10	0	0
Hv40	0	0		0	0	22	0	0	0	0	0	0	0	0	0
Hv41	30	24	0		30	12	22	0	0	0	0	10	23	25	20
Hf51	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Hf52	0	0	0	0	10		0	0	0	0	0	0	0	0	0
Hh5	0	0	0	10	0	10		0	0	0	10	0	0	0	0
Hh81	0	20	10	0	0	0	0		0	0	0	11	0	10	0
Hh82	0	0	0	0	0	0	0	0		0	0	0	0	0	0
Hh91	0	0	0	0	0	0	0	0	0		0	0	0	0	0
Hh92	0	0	0	0	0	0	0	0	0	0		0	0	0	0
Hh93	33	0	0	28	0	30	31	0	0	28	0		15	20	0
Hh94	0	0	12	0	0	0	0	0	0	0	0	0		0	0
Mm1	0	0	0	0	0	10	0	0	0	0	10	0	0		0
Mm3	0	10	0	0	0	10	25	0	0	0	10	0	0	15	
Mm31	0	0	0	0	0	10	0	0	0	0	10	0	0	0	0
Mm32	10	10	0	10	0	10	0	0	0	0	10	0	0	0	0
Mm33	0	12	0	10	0	10	0	0	0	0	10	0	0	0	0
Mm35	0	10	0	12	0	10	0	0	0	0	10	0	0	12	0
Mm51a	0	12	0	0	0	10	0	0	0	0	12	0	10	0	0
Mm51b	0	10	0	0	0	10	0	0	0	0	10	0	0	0	0
Mm81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm82	0	0	0	0	0	0	0	10	14	0	0	0	0	0	0
Mm84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mm91	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0
Pc31	0	10	0	0	0	10	0	0	0	0	10	0	10	0	20
Pc32	0	25	0	0	0	12	15	0	0	0	10	0	10	0	15
Pc5a	0	10	0	0	0	10	0	10	0	0	0	0	0	0	0
Pc5b	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
Pj1	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
Pj2	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
Pj32	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
Pj33	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
Pj52	0	10	0	0	0	10	0	0	0	0	0	0	0	0	0
Pm42	10	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Pm52	15	10	0	0	0	0	0	0	0	0	0	0	0	0	0

Ti41	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0
Ti54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ti55	10	0	0	10	0	10	10	0	0	0	10	0	0	10	0
Ti56	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0

Indicator	Mm32	Mm33	Mm35	Mm51a	Mm51b	Mm81	Mm82	Mm84	Mm91	Pc31	Pc32	Pc5a	Pc5b
Producer													
Pa51	0	0	0	0	10	0	0	0	10	0	10	0	10
Pa52	0	0	0	0	10	0	0	0	10	0	10	0	10
Pa53	0	0	0	0	10	0	0	0	10	0	10	0	10
Ca31	0	0	0	0	10	0	0	0	10	0	10	0	10
Cc81	0	0	0	0	0	0	0	0	10	0	0	0	0
Cc82	0	0	0	0	0	0	0	0	0	0	0	0	0
Cc92	0	0	0	0	10	0	0	10	0	0	0	0	0
Cc93	0	0	0	0	0	0	0	0	0	0	0	0	0
Cc94	18	25	0	0	0	0	0	0	0	20	20	20	0
Dr32	0	0	0	0	10	0	0	0	10	0	10	0	10
Dr34	0	0	0	0	10	0	0	0	10	0	12	0	10
Dr35	0	0	0	0	10	0	0	0	12	0	10	0	10
Dr36	0	0	0	0	10	0	0	0	21	0	10	0	10
Dr37	0	0	0	0	10	0	0	0	12	0	0	0	10
Dr5	0	0	0	0	10	0	0	0	10	0	0	0	10
Dr72	0	0	0	0	10	0	0	0	10	0	0	0	0
G51	0	0	0	0	10	10	10	30	20	0	15	0	10
G54	0	0	0	0	10	0	0	0	10	10	0	0	0
Hv40	0	0	0	0	0	0	0	0	10	0	0	0	0
Hv41	30	20	20	28	12	0	0	0	18	28	24	28	0
Hf51	0	0	0	0	0	0	0	0	0	0	0	0	0
Hf52	0	0	0	0	10	0	0	0	0	0	0	0	12
Hh5	0	0	0	10	10	0	0	0	15	0	10	12	10
Hh81	0	0	18	0	0	10	0	0	25	0	0	0	0
Hh82	0	0	0	0	0	0	0	0	10	0	0	0	0
Hh91	0	0	0	0	10	0	0	10	0	0	0	0	0
Hh92	0	0	0	0	0	0	0	10	0	0	10	0	0
Hh93	0	25	0	0	0	0	0	0	0	25	25	20	0
Hh94	0	0	0	0	10	10	10	10	0	0	0	0	0
Mm1	0	0	0	0	12	0	0	0	0	0	10	0	0
Mm3	15	13	0	21	15	0	0	0	11	22	0	28	25
Mm31	0	0	0	0	10	0	0	0	0	0	10	0	10
Mm32		0	0	0	10	0	0	0	0	0	10	0	10
Mm33	0		0	0	10	0	0	0	0	0	10	0	10
Mm35	23	20		21	10	0	0	0	10	0	0	0	10
Mm51a	0	0	0		0	0	0	0	11	0	0	0	10
Mm51b	0	0	0	0		0	0	0	10	0	10	0	10
Mm81	0	0	0	0	0		0	0	0	0	0	0	0
Mm82	0	0	0	0	10	15		12	12	0	0	0	0
Mm84	0	0	0	0	0	0	0		0	15	0	0	0
Mm91	0	0	0	0	10	0	0	0		0	0	0	0
Pc31	0	0	10	0	0	0	0	0	10		0	25	10
Pc32	0	0	0	15	20	0	0	0	10	12		0	11
Pc5a	0	0	10	0	0	0	0	0	10	0	0		0
Pc5b	0	0	10	0	0	0	0	0	10	0	11	0	
Pj1	0	0	10	0	0	0	0	0	10	0	0	0	10
Pj2	0	0	10	0	0	0	0	0	10	0	10	0	10
Pj32	0	0	10	0	0	0	0	0	10	0	10	0	10
Pj33	0	0	10	0	0	0	0	0	10	0	0	0	0
Pj52	0	0	10	0	0	0	0	0	0	0	10	0	10
Pm42	0	0	0	0	0	0	0	0	0	0	0	0	0

Pm52	0	0	0	0	0	10	0	10	0	0	0	0	0
Ti41	0	0	0	0	10	0	0	10	0	0	10	0	0
Ti54	0	0	0	0	0	0	12	15	0	0	0	0	0
Ti55	0	0	0	0	0	0	10	10	0	0	0	0	0
Ti56	0	0	0	0	0	0	0	0	0	0	10	0	0

Indicator	Pj1	Pj2	Pj32	Pj33	Pj52	Pm42	Pm52	Ti41	Ti54	Ti55	Ti56
Producer											
Pa51	10	10	0	0	0	0	0	10	0	10	0
Pa52	10	10	0	0	0	0	0	10	0	10	0
Pa53	10	10	0	0	0	0	0	10	0	10	0
Ca31	10	10	0	10	0	0	0	10	0	10	0
Cc81	0	0	0	0	0	0	0	0	0	0	0
Cc82	0	0	0	0	0	0	0	0	0	0	0
Cc92	0	10	0	0	0	0	0	10	0	0	0
Cc93	0	0	0	0	0	0	0	0	0	0	0
Cc94	0	0	10	10	10	0	10	10	25	0	0
Dr32	10	10	0	0	0	0	0	10	0	10	0
Dr34	10	0	0	0	0	0	0	10	0	10	10
Dr35	0	0	0	0	0	0	0	18	0	0	10
Dr36	10	10	0	0	0	0	0	10	0	10	10
Dr37	10	10	0	0	0	0	0	18	0	10	13
Dr5	10	10	0	0	0	0	0	27	0	10	0
Dr72	10	10	0	0	0	0	0	10	0	10	0
G51	10	10	0	0	10	0	0	10	10	20	0
G54	0	0	0	0	0	0	0	10	0	10	0
Hv40	0	0	0	0	0	0	0	0	0	10	0
Hv41	0	0	0	24	0	10	0	10	10	0	20
Hf51	0	0	0	0	0	0	0	0	0	0	0
Hf52	10	10	0	0	0	0	0	10	0	10	10
Hh5	10	10	0	0	0	0	0	10	0	10	0
Hh81	0	0	0	0	0	0	0	0	0	10	0
Hh82	0	0	0	0	0	0	0	0	0	0	0
Hh91	0	0	0	0	0	0	0	0	0	0	0
Hh92	20	11	20	0	0	18	0	0	0	15	0
Hh93	0	0	10	10	10	25	0	15	26	10	0
Hh94	0	0	0	0	0	0	0	0	0	0	0
Mm1	10	10	0	0	0	0	0	11	0	10	0
Mm3	16	10	0	14	0	15	0	10	0	21	0
Mm31	10	10	0	0	0	0	0	0	0	0	0
Mm32	10	10	0	0	0	0	0	0	0	0	0
Mm33	10	10	0	0	0	0	0	0	0	0	0
Mm35	10	10	0	0	0	0	0	0	0	0	0
Mm51a	10	10	0	0	0	0	0	0	0	0	0
Mm51b	10	10	0	0	0	0	0	0	0	0	0
Mm81	0	0	0	0	0	0	0	0	0	0	0
Mm82	0	12	12	0	0	0	0	0	0	12	0
Mm84	0	0	0	0	0	0	0	0	0	0	0
Mm91	0	0	0	0	0	0	0	0	0	0	0
Pc31	10	10	0	0	0	0	0	10	0	0	0
Pc32	22	10	12	0	17	0	0	10	0	0	0
Pc5a	10	10	0	0	0	0	0	0	0	10	10
Pc5b	10	10	0	0	0	0	0	0	0	10	10
Pj1		10	0	0	0	0	0	0	0	0	0
Pj2	10		0	0	0	0	0	10	0	10	10
Pj32	10	10		0	0	0	0	0	0	0	0
Pj33	10	10	0		0	0	0	0	0	0	0
Pj52	10	0	0	0		0	0	0	0	0	0

Pm42	10	0	0	0	0		0	10	0	0	0
Pm52	0	0	0	0	0	0		0	0	0	0
Ti41	0	0	0	0	0	0	0		0	0	0
Ti54	0	0	0	0	0	0	0	0		0	0
Ti55	0	0	10	0	0	0	0	0	0		0
Ti56	0	0	0	0	0	0	0	0	0	0	

[a]The strains belong to the sponge-associated bacteria collection of the Molecular and Marine Bacteriology Laboratory (MMBL) of the Microbiology Institute, UFRJ, Brazil.
[b]Diameter of the inhibition zones in millimeters of each interaction.

Table 2: Antagonistic interactions among sponge-associated bacteria from the Brazilian coast.

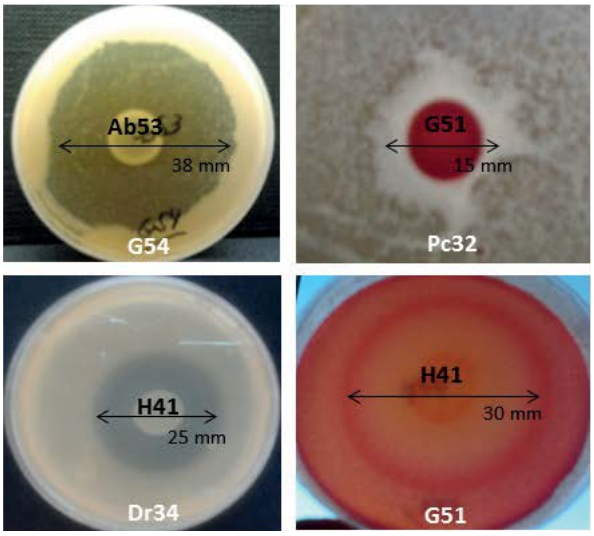

Figure 1: Some examples of antagonistic activity assays with bacterial isolates from sponges. Active strains are labeled in black while sensitive strains are labeled in white. The diameter of the inhibition zone (arrow) is measured in millimeters (mm).

activity. *Bacillus, Klebsiella* and *Psychrobacter* were involved in antagonistic interactions as both, active and sensitive strains in a similar proportion. However, some bacterial genera included a higher number of active than sensitive strains (like *Brachybacterium*, *Kocuria, Pseudomonas*, *Pseudovibrio* and *Serratia*), while others included more sensitive than active strains (*Brevibacillus*, *Enterococcus*, *Lactococcus* and *Rhodococcus*) (Figure 4).

Cross-inhibition among isolates associated with different species of sponges collected at the same site and on the same date

Under this condition, relative frequencies of antagonistic interactions among bacteria ranged from 5.5 to 50.0% (Table 3). The weighted mean of all frequencies was 17.7%, a value very close to that (18%) observed among the 56 isolates. Therefore, for a given site at a given sampling date, bacteria isolated from different sponge species cross-inhibited their growth at a frequency that was comparable to the situation in which all strains were tested.

When isolates were classified into three interactivity clusters, 60% of the strains were resistant in their relationships, followed by 40% of the sensitive strains and finally 32% of the active strains. The resistant cluster includes strains that are either exclusively resistant or both, resistant and active; the sensitive cluster includes strains that are either exclusively sensitive, or sensitive and active; and the active cluster includes strains which proved to be either active and sensitive or active and resistant.

Cross-inhibition among isolates associated with the same sponge species collected at different sites and on different dates

The frequency of antagonistic interactions was also analyzed among isolates from the same sponge species which were spatially separated by about 10 km along a pollution gradient. In this condition, 17.5% of the interactions were inhibitory. This percentage is similar to those measured in the aforementioned analyses. The majority of isolates were classified as resistant, except for isolates from the sponge *M. microsigmatosa* (Mm), where the active cluster was predominant (Table 4).

Cross-inhibition among isolates associated with the same species of sponge collected at the same site but in different years

Relationships among bacteria obtained from the same sponge species collected at the same site over 6 years (2005-2011) showed a relative frequency of antagonistic interactions ranging from 0.0 to 50.0% (Table 5). The results were very different among sponge-associated bacteria, and the lowest percentage was observed among the isolates from site Us.

Cross-inhibition among isolates associated with the same sponge specimen

Few bacteria inhibited isolates from the same sponge specimen, especially among those from sponges collected at the CA site (Table

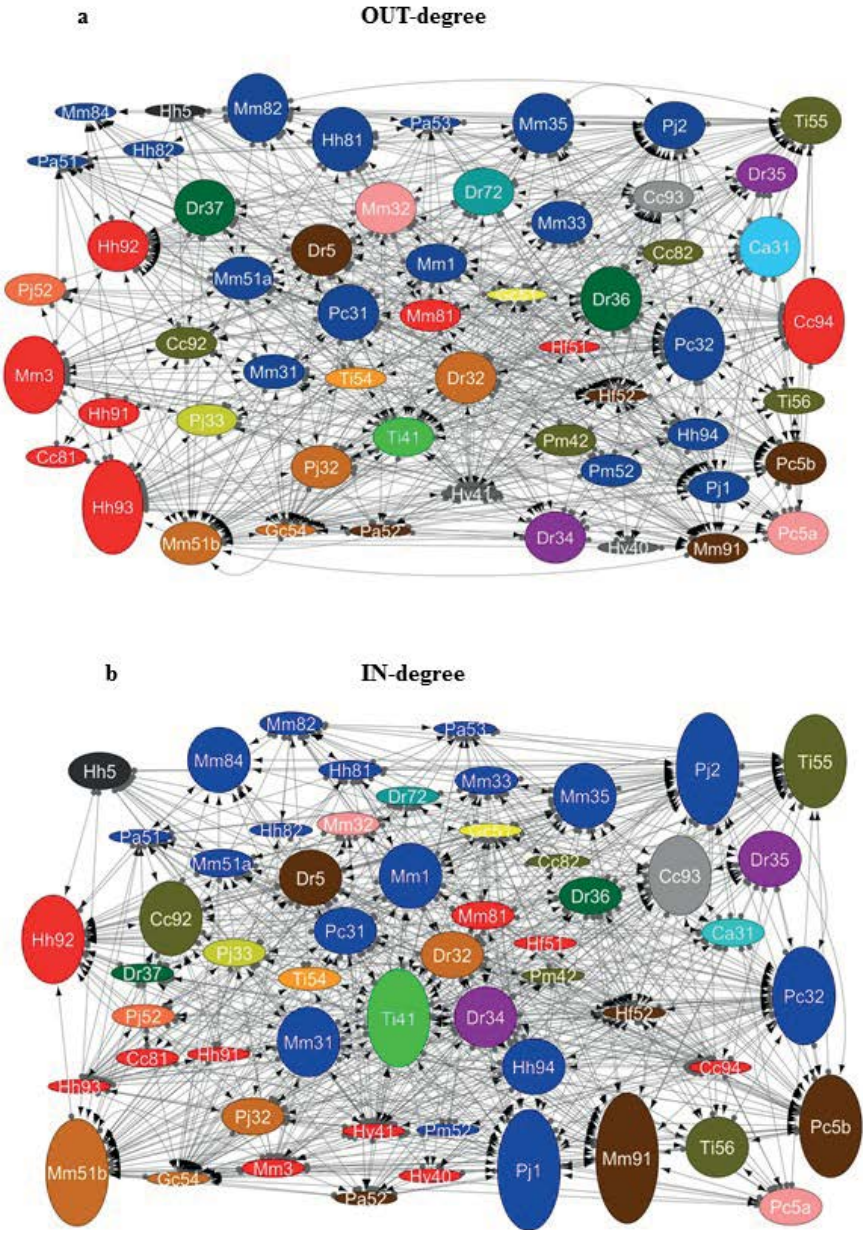

Figure 2: Network analysis of antagonistic interactions among sponge-associated bacteria. A 56x55 array of tests was performed and the results were converted to graphs using the Cytoscape 3.1.0 software (http://www.cytoscape.org). Each node represents a bacterial strain. Each line (connection) represents an antagonistic interaction from an active strain (grey dot) towards a sensitive strain (arrow). Strains isolated from the same bacterial genus have the same fill color. Strains isolated from the same sponge species have the same initial letters: Ca (*Clathrina aurea*), Cc (*Cliona aff. Celata*), Dr (*Dragmacidon reticulatum*), Gc (*Geodia corticostylifera*), Hv (*Haliclona vansoesti*),Hf (*Haliclona fugidia*), Hh (*Hymeniacidon heliophila*), Mm (*Mycale microsigmatosa*),Pa (*Pachychalina alcaloidifera*),Pm (*Paraleucilla magna*),Pc (*Petromica citrina*), Pj (*Polymastia janeirensis*),Ti (*Tedania ignis*). Node size is proportional to the number of antagonistic interactions with other isolates: in (a) to the number of connections leaving the node (strain activity) ("out-degree") and in (b) to the number of connections reaching the node (strain sensitivity) ("in-degree").

6). Relative frequencies of antagonism were closer between the bacteria from Us and PV sites. In addition, the majority of isolates from CA site was classified in the resistant interactivity cluster, (i.e. resistant and active strains, as well as exclusively resistant strains). However, the distribution of the isolates from PV and Us was similar among the three interactivity clusters.

Interestingly, sponge-associated bacteria which were active against numerous strains when all were cross-tested (see previous results) and showed no inhibitory activity against bacterial isolates from the same sponge. This was the case for strains H41 and Hh93 which inhibited 64% and 50% of all 55 tested bacteria, respectively (Table 2).

Discussion

Bacteria from a wide range of marine environments, including sediments, seawater, biofilms, and tissues/surfaces of invertebrate and algae, have been shown to possess antagonistic activities. In most cases, these bacteria are members of complex communities in which competition for limited space and resources can be intense [19].

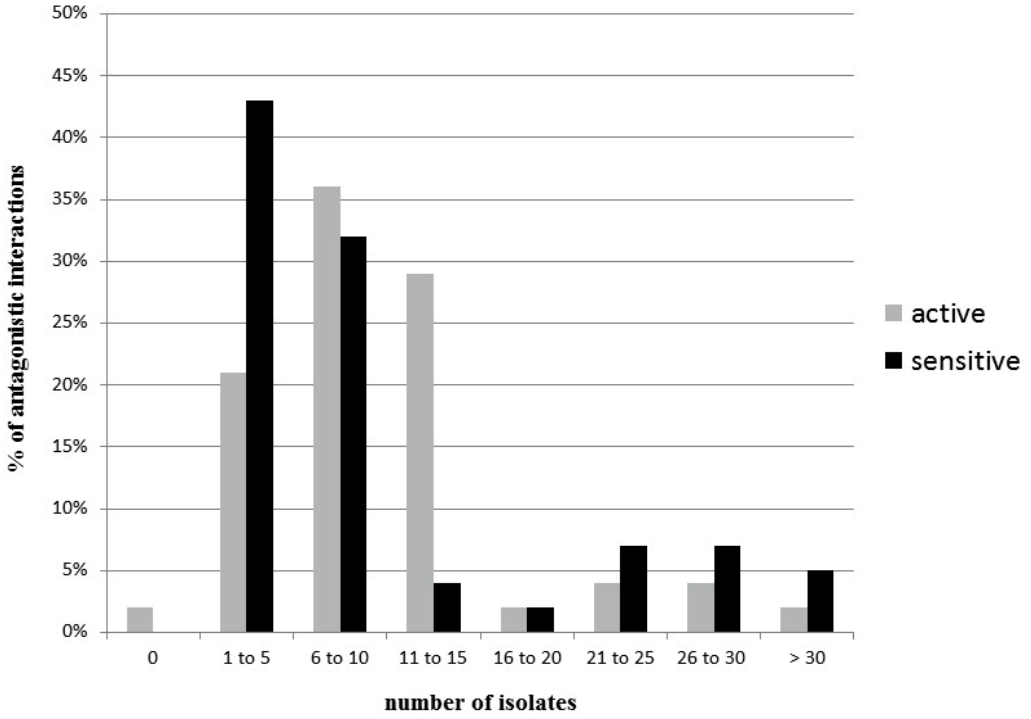

Figure 3: The relative frequency of antagonistic interactions among sponge-associated bacteria. Bacterial strains were operationally distinguished into three different interactivity clusters, termed: (I) active, if they were able to inhibit the growth of at least one indicator strain; (II) sensitive, if their growth was inhibited by at least one producer strain; and (III) resistant if their growth was never inhibited by any producer strain.

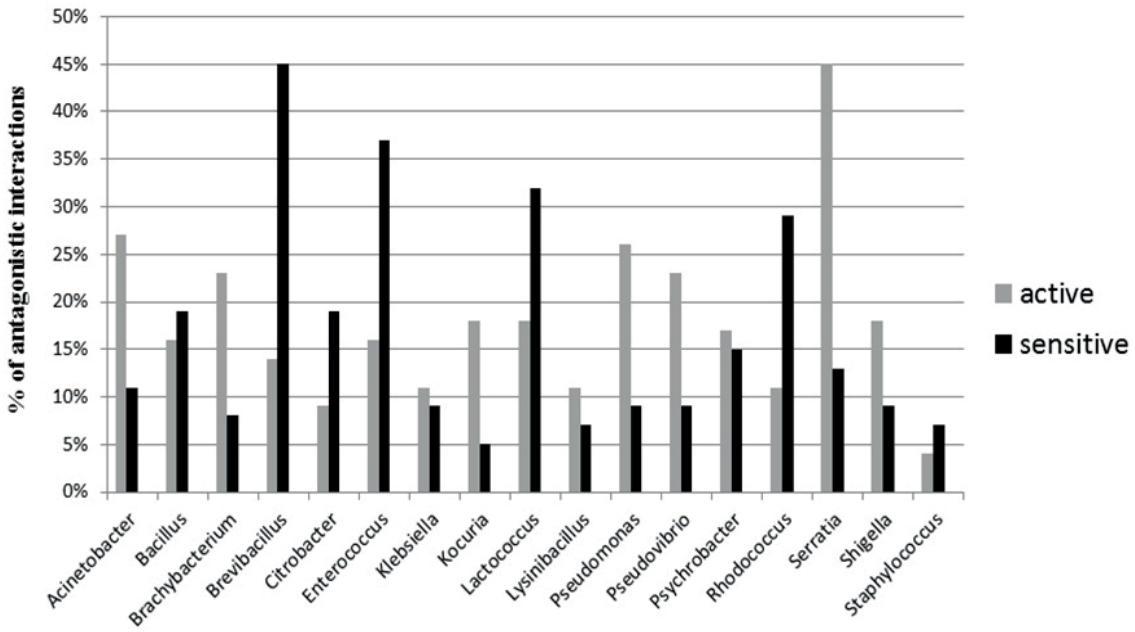

Figure 4: Relative frequency of antagonistic interactions among sponge-associated bacteria grouped according to genus affiliation. Separate results are presented for bacteria classified as “active” or “sensitive” in the cross-inhibition tests.

Antagonistic interactions may play an important role in structuring these communities, where the evolutionary advantages afforded by an effective chemical defense may be crucial for survival.

The bacterial isolates belonged to the Firmicutes, Gammaproteobacteria, Actinobacteria and Alphaproteobacteria classes. These bacterial groups represented the predominant cultivable sponge-associated bacteria reported in other studies [2,3,20,21]. It is known that many bacterial inhabitants in sponges appear to be

Site / year	Sponges	Frequencies	Interactivity clusters		
		absolute (relative)	I	II	III
PV/2005	Mm, Pj	4 (50.0%)	2	2	2
PV/2006	Ca, Dr, Mm, Pj	3 (5.5%)	3	2	10
PV/2007	Dr, Hf, Hh, Pj	2 (11.1%)	2	1	4
CA/2007	Gc, Mm, Pa, Pc, Pm, Ti	31 (22.5%)	12	7	6
CA/2008	Hv, Pm, Ti	3 (30.0%)	2	2	2
Us/ 2010	CC, Hh, Mm	4 (12.5%)	2	4	3
Us/2011	CC, Hh, Mm	6 (15.8%)	5	3	5
Weighted mean	17.7%				

Cagarras Archipelago (CA), Praia Vermelha beach (PV), Urca square (Us). Sponge species: *Clatrina aurea* (Ca), *Cliona aff. celata* (Cc), *Dragmacidon reticulatum* (Dr), *Geodia cortiscolylifera* (G), *Haliclona fugidia* (Hf), *Haliclona vansoesti* (Hv), *Hymeniacidon heliophila* (Hh), *Mycale microsigmatosa* (Mm), *Pachychalina alcaloidifera* (Pa), *Petromica citrina* (Pc), *Paraleucilla magna* (Pm), *Polymastia janeirensis* (Pj), *Tedania ignis* (Ti). Number of isolates classified into three different interactivity clusters, termed: (I) active, (II) sensitive and (III) resistant strains.

Table 3: Cross-inhibition among bacteria isolates from different sponge species collected from the same site and date.

Voucher	Sponge	Site / year	Frequencies	Interactivity clusters		
			absolute (relative)	I	II	III
62009Dr	Dr	Ca/2009	1 (8.3%)	1	1	6
42006Dr		PV/2006				
32007Dr		PV/2007				
12007Hv	H	Ca/2007	3 (37.5%)	2	2	2
32007Hf		PV/2007				
32007Hh	Hh	PV/2007	2 (16.6%)	2	2	5
92010Hh		Us/2010				
82011Hh		Us/2011				
82005Mm	Mm	Pv/2005	15 (17.0%)	10	4	8
42006Mm		Pv/2006				
92010Mm		Us/2010				
82011Mm		Us/2011				
Weighted mean	17.50%					

Cagarras Archipelago (CA), Praia Vermelha beach (PV), Urca square (Us). Sponge: *Dragmacidons reticulatum* (Dr), *Haliclona* sp. (H), *Hymeniacidon heliophila* (Hh), *Mycale microsigmatosa* (Mm). Number of isolates classified into three different interactivity clusters, termed: (I) active, (II) sensitive and (III) resistant strains.

Table 4: Cross-inhibition among bacteria isolated from the same sponge collected from different sites and dates.

Sponge	Site / years	Frequencies	Interactivity clusters		
		absolute (relative)	I	II	III
Pc	CA/ 2006, 2007	4 (50.0%)	3	3	1
Pm	CA/ 2007, 2008	0 (0.0%)	0	0	2
Ti	CA/ 2007,2008	0 (0.0%)	0	0	4
Dr	Pv/ 2006, 2007, 2009	1 (10.0%)	1	1	5
Mm	Pv/ 2005, 2006	4 (25.0%)	2	4	2
Pj	Pv/ 2005, 2006, 2007	5 (31.2%)	3	2	3
Cc	Us/ 2010, 2011	0 (0.0%)	0	0	0
Hh	Us/ 2010, 2011	1 (6.2%)	1	1	5
Mm	Us/ 2010, 2011	1 (16.7%)	1	1	3

Cagarras Archipelago (CA), Praia Vermelha beach (PV), Urca square (Us). Sponge species: *Cliona aff. celata* (Cc), *Dragmacidon reticulatum* (Dr), *Hymeniacidon heliophila* (Hh), *Mycale microsigmatosa* (Mm), *Petromica citrina* (Pc), *Paraleucilla magna* (Pm), *Polymastia janeirensis* (Pj), *Tedania ignis* (Ti). Number of isolates classified into three different interactivity clusters, termed: (I) active, (II) sensitive and (III) resistant strains.

Table 5: Cross-inhibition among bacteria isolates from the same sponge species collected from the same sites and in different years.

recalcitrant to cultivation on laboratory media, probably reflecting their evolutionary adaptation to the conditions provided by the host [2]. Therefore, the classical culture dramatically underestimates microbial numbers and diversity found in the samples under study. However, the major advantage of this approach over modern molecular techniques is that it provides the researcher with live microbes, which can be used in further studies [22], such as the present one.

This study is the first to analyze the antagonistic interactions among 56 sponge-associated bacteria. Bacteria belonging to all 17 cultured genera showed inhibitory activity. Our data demonstrate that the antagonistic interaction is present among the isolates and expression of this phenotype depends on both the identity of the producer strain and that of the indicator strain. In contrast to terrestrial environments, which are essentially static, the marine environment also involves dispersion and movement of communities driven by hydrography, thus complicating the interpretation of results [23].

Voucher number	Sponges	Site / year	Number of bacteria	Frequencies absolute (relative)	Interactivity clusters		
					I	II	III
32007Gc	Gc	CA / 2007	2	1 (50.0%)	1	1	1
12007Hv	Hv	CA / 2007	2	0 (0.0%)	0	0	2
32007Mm	Mm	CA / 2007	2	0 (0.0%)	0	0	2
32007Pa	Pa	CA / 2007	3	0 (0.0%)	0	0	3
42006Pc	Pc	CA / 2006	2	1 (50.0%)	1	1	1
32007Pc	Pc	CA / 2007	2	0 (0.0%)	0	0	2
32007Ti	Ti	CA / 2007	3	0 (0.0%)	0	0	3
42006Dr	Dr	Pv / 2006	5	0 (0.0%)	0	0	0
42006Hf	Hf	Pv / 2007	2	1 (50.0%)	1	1	1
82005Mm	Mm	Pv / 2005	4	2 (16.7%)	1	2	2
42006Mm	Mm	Pv / 2006	2	1 (50.0%)	1	1	1
52005Pj	Pj	PV / 2005	2	2 (100.0%)	2	2	0
42006Pj	Pj	PV / 2006	2	0 (0.0%)	0	0	2
92010Cc	Cc	Us / 2010	2	0 (0.0%)	0	0	0
82011Cc	Cc	Us / 2011	3	0 (0.0%)	0	0	0
92010Hh	Hh	Us / 2010	2	0 (0.0%)	0	0	2
82011Hh	Hh	Us / 2011	4	2 (16.7%)	1	2	2
92010Mm	Mm	Us / 2010	3	2 (33.3%)	1	2	1

Cagarras Archipelago (CA), Praia Vermelha beach (PV), Urca square (Us). Sponge species: *Cliona aff. celata* (Cc), *Dragmacidon reticulatum* (Dr), *Geodia cortiscotylifera* (Gc), *Haliclona fugidia* (Hf), *Haliclona vansoesti* (Hv), *Hymeniacidon heliophila* (Hh), *Mycale microsigmatosa* (Mm), *Pachychalina alcaloidifera* (Pa), *Petromica citrina* (Pc), *Paraleucilla magna* (Pm), *Polymastia janeirensis* (Pj), *Tedania ignis* (Ti). Number of isolates classified into three different interactivity clusters, termed: (I) active, (II) sensitive and (III) resistant strains.

Table 6: Cross-inhibition among bacteria isolated from the same sponge specimen.

All strains used in this study were previously analyzed for their production of antimicrobial substances against bacteria of medical importance [15,18]. Overall, the percentage of active bacteria found in this study (98.2%) was much higher than those (22%) reported for the same isolates against clinical pathogenic bacteria. We suggest that the enhanced production of bioactive compounds may occur to inhibit other sponge bacteria, i.e. potential *in situ* competitors for nutrients and space [24]. Clinical pathogenic bacteria are not natural competitors for sponge-associated bacteria and therefore lack the secondary metabolites that would induce the antimicrobial activity [25].

It is relevant to note that secondary metabolites from marine bacteria are involved in a variety of processes, including nutrient acquisition [26] and chemical communication [27]. Mechanisms responsible for antagonistic effects can vary widely, ranging from direct cell killing, as in the case of an antibiotic, to the removal of an essential nutrient, as in the case of an iron chelating siderophore. Antagonism also can result from the production of small organic acids or other compounds that render the environment unsuitable for growth of competing bacteria [28]. These compounds likely play important ecological roles that ultimately affect ecosystem structure and function; however, much remains to be discovered before these processes can be fully appreciated [29].

Temporal and spatial variability of sponge bacterial communities has been discussed and some researchers have shown that there are differences among bacterial communities across sponge species or even specimens [30,31]. Indeed marine sponges are well known for their associations with highly diverse, yet very specific and often highly similar microbiota [2]. Furthermore, sponges filter large amounts of water and can collect contaminants from both dissolved and particulate phases [32]. The nature of accumulation between different sponge species may be related to composition of symbiotic microorganism communities, skeletal composition, histology, and life cycle [14]. This can be seen with the data obtained in this study, where no antagonistic activity was observed among isolates from *C. celata* (Us site, 2010-2011), *P. magna* (CA site, 2007-2008) and *T. ignis* (CA site, 2007-2008) sponges, whereas isolates from *P. citrina* (CA site, 2006/2007) were the most active, including among themselves. This data was not surprising, since bacterial communities are dynamic in respect of responding to environmental conditions. Moreover, seasonal changes in the production of bioactive compounds by sponges may be considered [33]. Sponges therefore contain a uniform, sponge-specific bacterial community, although each sponge species contains different bacterial species [34]. Recent advances in studying the dynamics of marine bacterial communities have shown that the composition of these communities follows predictable patterns and involves complex network interactions, which shed light on the underlying processes regulating these globally important organisms [35].

It is important to point out that the Guanabara Bay, in the Rio de Janeiro state, Southeast Brazil, is one of the largest and most polluted estuaries on the Brazilian coast [36]. CA integrates a protected marine area situated approximately 8 km southwest from the entrance of the Guanabara Bay. These islands are important areas for fishery and tourism and receive alternating influence from clean waters from oceanic currents and polluted waters from coastal discharges. The polluted waters from Guanabara Bay also have some influence in this area, which are, however, much less polluted than the center of the bay [14]. The collection sites are therefore located along a marked environmental gradient, from highly polluted (Us) to moderately (PV) and slightly polluted sites (CA).

Our study traced the broad profile of antagonistic interactions among sponge-associated bacteria isolated from the same or different sponge species, sites and years. However, in order to establish a connection between an antagonistic activity observed in the laboratory and an ecologically meaningful effect, many factors must be considered, such as the biogeography, seasonal variation and environmental factors. Future studies will be conducted with greater control over

these variables. Overall, our study demonstrates that antagonism could be a structuring force in sponge-associated microbial communities.

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