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## Antimicrobial activity of Red Sea corals

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**Abstract** Scleractinian corals and alcyonacean soft corals are the two most dominant groups of benthic marine organisms inhabiting the coral reefs of the Gulf of Eilat, northern Red Sea. Antimicrobial assays performed with extracts of six dominant Red Sea stony corals and six dominant soft corals against marine bacteria isolated from the seawater surrounding the corals revealed considerable variability in antimicrobial activity. The results demonstrated that, while the majority (83%) of Red Sea alcyonacean soft corals exhibited appreciable antimicrobial activity against marine bacteria isolated from the seawater surrounding the corals, the stony corals had little or no antimicrobial activity. From the active soft coral species examined, *Xenia macrospiculata* exhibited the highest and

most potent antimicrobial activity. Bioassay-directed fractionation indicated that the antimicrobial activity was due to the presence of a range of compounds of different polarities. One of these antibiotic compounds was isolated and identified as desoxyhavannahine, with a minimum inhibitory concentration (MIC) of  $48 \mu\text{g ml}^{-1}$  against a marine bacterium. The results of the current study suggest that soft and hard corals have developed different means to combat potential microbial infections. Alcyonacean soft corals use chemical defense through the production of antibiotic compounds to combat microbial attack, whereas stony corals seem to rely on other means.

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### Introduction

Bacteria and other microorganisms are ubiquitous in the marine environment. They are taxonomically diverse, biologically active, and colonize all marine habitats, from the deep oceans to the shallowest estuaries (Austin 1988; Rheinheimer 1992), as well as coral reefs (Ducklow 1990). The surface of living corals is covered by mucus (Ducklow and Mitchell 1979a). This mucus layer is colonized by bacteria, allowing for the establishment of a bacterial community that can be characteristic to a particular coral species (Mitchell and Chet 1975; Ducklow and Mitchell 1979b; Rublee et al. 1980; Segel and Ducklow 1982; Ritchie et al. 1994; Rohwer et al. 2002). Some of these bacteria can be pathogenic to corals, and may initiate disease, such as black band disease (Antonius 1985; Carlton and Richardson 1995), white plague type II (Smith et al. 1996; Richardson et al. 1998), tissue necrosis (Hodgson 1990; Ben-Haim and Rosenberg 2002), and even bleaching of the Mediterranean scleractinian coral *Oculina patagonica* (Kushmaro et al. 1996, 1997). On the other hand, bacteria could serve as beneficial symbionts or as benign associates. For example, Gil-Turnes et al. (1989) showed that bacteria on the surface of externally held eggs of the shrimp

*Palaeman macrodactylus* produce a metabolite that inhibits fungal infections that are lethal to the eggs. Therefore, corals need the ability to regulate the bacteria they encounter and to resist microbial colonization and the invasion of potential pathogens, in order to prevent possible detrimental effects.

Corals are able to hinder unwanted bacteria by several means, such as the self-cleaning of mucus from their surface (Ducklow and Mitchell 1979b). Another potential method is the maintenance of antimicrobial chemical defenses targeted at pathogens or other potentially deleterious microorganisms. Antimicrobial activity has been extensively reported for extracts of various groups of marine organisms, such as sponges (Burkholder and Ruetzler 1969; Bergquist and Bedford 1978; Amade et al. 1982; McCaffrey and Endean 1985; Amade et al. 1987; Becerro et al. 1994; Newbold et al. 1999; Kelman et al. 2001), bryozoans (Walls et al. 1993), ascidians (Wahl et al. 1994), scleractinian corals (Koh 1997), scleractinian coral eggs (Marquis et al. 2005), gorgonian octocorals (Burkholder and Burkholder 1958; Kim 1994; Jensen et al. 1996), and alcyonacean soft corals (Slattery et al. 1995; Kelman et al. 1998). Several antibiotics have been isolated, such as plakortin (Higgs and Faulkner 1978), manoalide (De Silva and Scheuer 1980), and halitoxin (Kelman et al. 2001) from marine sponges, and sinulariolide and flexibilide (Aceret et al. 1998) from alcyonacean soft corals.

Many of the reports on antimicrobial activity of extracts of marine organisms and the subsequent purified antibiotics isolated from these organisms were tested against human pathogens as potential novel clinically useful drugs. Activity was tested and found mainly in marine sponges and gorgonian octocorals. Little is known on the antimicrobial activity of other corals, especially reef-building (hermatypic) stony (scleractinian) corals. This is rather surprising, considering that these latter organisms are the most dominant and conspicuous members of many reefs. Scleractinian corals and alcyonacean soft corals are the two most dominant groups of benthic marine organisms inhabiting the coral reefs of the Gulf of Eilat, Red Sea (Benayahu and Loya 1977). Therefore, the aim of the current study was to compare the antimicrobial activity of extracts of several of the most dominant stony and soft coral species from the coral reef of Eilat (northern Red Sea) against bacteria isolated from the waters surrounding the corals.

## Materials and methods

### Collection

Six species of stony (scleractinian) corals and six species of soft (alcyonacean) corals were collected using SCU-BA from the coral reef of Eilat (northern Red Sea) between April 1998 and March 2001 at depths of 1–10 m. Attempts were made to collect the most abundant species observed. In most cases, three replicate colonies of each species were collected. All corals collected were identified to species level. Each coral sample was immediately frozen after collection and maintained at  $-20^{\circ}\text{C}$  prior to extraction.

Samples for bacterial isolation were collected from seawater at the same location. The samples were collected *in situ* by opening 50-ml sterile tubes near the coral colonies. All samples were kept at  $4^{\circ}\text{C}$  and transferred immediately to the laboratory in Tel-Aviv for bacterial isolation.

### Bacterial isolation, characterization, and identification

The bacteria used in the antimicrobial assays (Table 1) were isolated using standard serial dilution and plating techniques on Marine Agar [18 g Difco Marine Broth (MB), 9 g NaCl and 18 g Difco Agar, per 1 l of deionized water], and incubated at  $25^{\circ}\text{C}$ , corresponding to the ambient seawater temperature.

Sensitivity to antibiotics (10  $\mu\text{g}$  penicillin-G, 10  $\mu\text{g}$  ampicillin, 30  $\mu\text{g}$  kanamycin, 30  $\mu\text{g}$  tetracycline, and 15  $\mu\text{g}$  erythromycin, each applied to a paper disc) was determined after incubation for 24 h at  $25^{\circ}\text{C}$  on Marine Agar.

The bacteria used in the antimicrobial assays were identified by 16S ribosomal RNA (rRNA) sequence analysis or by the BIOLOG kit. For the identification by 16S rRNA sequence analysis, the bacterial isolates were grown overnight in 2 ml MB. Total DNA was extracted with the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) using the manufacturer procedure. Eubacterial-specific primers [forward primer 8–27: 5'-AGAGTTTGATCCTGGCT-CAG-3' (Weisburg et al. 1991) and reverse primer 1492: 5'-GGTTACCTTGTACGACTT-3' (Reysenbach et al.

**Table 1** Identification of Red Sea seawater bacteria used in the antimicrobial assays

Bacterial strain	Genbank accession number	Closest relative in the database <sup>a</sup>	Similarity (%)	Bacterial group	Method of identification
RSW-2	DQ110006	<i>Arthrobacter</i> sp. KP17	99%	Actinobacteria	16S rRNA
RSW-3	DQ110008	<i>Arthrobacter</i> sp. Muzt-E04	99%	Actinobacteria	16S rRNA
RSW-17	n.a. <sup>b</sup>	<i>Vibrio metschnikovii</i>	99%	Gamma-proteobacteria	BIOLOG
RSW-18	DQ110007	<i>Vibrio</i> sp. 1B07	100%	Gamma-proteobacteria	16S rRNA

<sup>a</sup>Genbank database for 16S rRNA sequence analysis and BIOLOG (Hayward, CA, USA) bacterial database for BIOLOG analysis

<sup>b</sup>Not applicable

1992)] were used to amplify 16S rRNA genes. PCR fragments were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA), and sequenced on an ABI 377 automated sequencer using the PRISM Ready Reaction Kit (Applied BioSystems, Foster City, CA, USA). Sequence data were analyzed by comparison to 16S rRNA genes in the Genbank database. The nearest relatives of each organism were obtained by BLAST searches (Altschul et al. 1990). For the identification by the BIOLOG kit, the bacterial isolates were grown overnight in 125 ml MB and applied on the BIOLOG's GN2 and GP2 MicroPlates™ (Biolog Inc., Hayward, CA, USA). These test panels provide a standardized micromethod utilizing 95 different carbon sources for the identification of a broad range of gram-negative and gram-positive bacteria, respectively. The standard BIOLOG procedure was used, with the inoculating fluid adjusted to 2.5% NaCl, 0.8% MgCl<sub>2</sub>, 0.05% KCl, and 0.15% Carrageenan type II (Sigma, St. Louis, MO, USA) for marine bacteria.

#### Extraction and isolation

Prior to extraction the volume of the soft corals and the area of the tissue of stony corals were measured in order to calculate the natural (whole-tissue) concentration of the crude extract. For soft corals, samples were allowed to thaw, cut into small pieces, and then placed in a 1,000 ml graduated cylinder containing a known amount of seawater in order to measure their volume. The samples, drained of excess water, were extracted in a 1:1 (v/v) dichloromethane:methanol (DCM:MeOH) solution for 24 h at room temperature. For stony corals, samples were wrapped with aluminum foil to measure the area of the tissue that coats the coral skeleton. Then, the areas of the pieces of the aluminum foil were measured using an image analyzer. The coral samples were then freeze dried and extracted in a 1:1 (v/v) DCM:MeOH solution with 2% deionized water for 24 h at room temperature. The organic extracts were filtered, and the solvent was removed by rotary evaporation under vacuum at 30°C. The dried extracts were weighed and kept at -20°C for further use. For every sample, the natural (whole-tissue) concentration was recorded and applied later in the antimicrobial assays.

Preliminary investigations indicated that the extract of *Xenia macrospiculata* possessed the highest antimicrobial activity among the Red Sea corals investigated. Therefore, a larger amount of the coral tissue (13.2 g dry weight) was extracted as described above. The solvents were then evaporated to dryness under reduced pressure with a rotary evaporator, and the resulting extract was assayed for antimicrobial activity using the marine bacterium ST-1 (see Kelman et al. 2001 for details) as a guide throughout the purification process. The crude extract was fractionated by solvent partitioning with petrol ether (PE), DCM, and *n*-butanol as solvent

systems against aq. MeOH (10–20% H<sub>2</sub>O). The resulting fractions were evaporated to dryness and assayed for antimicrobial activity. Subsequent purification of constituent compounds from the active DCM phase employed size-exclusion chromatography using Sephadex LH-20 (Pharmacia, NJ, USA), with a 2:1:1 (v/v) PE:chloroform:MeOH elution. The most active fraction was further fractionated by silica vacuum liquid chromatography (VLC), eluting with solvents of increasing polarity (from 100% PE to 100% ethyl-acetate). A similar VLC column was then applied to one of the active fractions from the previous chromatography column and the bioassay-directed fractionation was continued until a purified active antimicrobial compound was successfully isolated. Resulting fractions were analyzed using thin-layer chromatography that was run with a 1:1 (v/v) PE:ethyl-acetate solvent system and developed with a vanillin-sulphuric acid solution and heated. The purified fractions were also subjected to antimicrobial assays. The active fractions were analyzed by <sup>1</sup>H-NMR (<sup>1</sup>H-nuclear magnetic resonance) using a Bruker ARX 500 NMR spectrometer. An active metabolite was finally purified by crystallization in a mixture of *n*-heptane and acetone. Comparison of the NMR data (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR, DEPT, HMBC, HMQC, COSY, and TOCSY) with literature values enabled structural elucidation of the active metabolite.

#### Antimicrobial assays

Assays were performed as previously described (Kelman et al. 1998). In brief, inocula of overnight cultures (approximately 10<sup>8</sup> cells per ml) of each bacterial strain were streaked onto the surface of Marine Agar plates. The coral extract dissolved in ethanol was adjusted to the same volumetric concentration as was present in the coral tissue (see above, extraction and isolation). Then, 30 µl of the extract was pipetted onto a 6 mm sterile paper disc, the solvent was allowed to evaporate, and the disc was placed on the surface of the inoculated agar. For every sample, duplicate discs were tested. The test plates were incubated for 24 h at 25°C. Solvent controls were performed in each case. Areas of inhibited bacterial growth were observed as clear halos (zones) around the discs. Antibacterial activity was measured as the mean diameter of zone of inhibition, excluding the paper disc diameter, for the duplicate discs of every sample.

Minimum inhibitory concentration (MIC) was measured by determining the smallest amount of extract or pure compound needed to inhibit the growth of a test bacterium. A series of tubes were filled with one milliliter of liquid broth medium containing a series of concentrations of extract or pure compounds (ranging from 0.6 to 480 µg ml<sup>-1</sup>), test bacteria, and solvent controls. After overnight incubation at 25°C, the tubes in which growth did not occur were noted.

## Results

Four strains of bacteria were isolated from samples of seawater surrounding the corals. Their identifications are summarized in Table 1. The strains were identified by 16S rRNA sequence analysis, except strain RSW-17, which was identified by the biochemical micromethod BIOLOG. This was done since multiple amplification and sequencing attempts did not provide suitable sequence for analysis and identification. These strains also exhibited variable sensitivity to different commercial antibiotics (results not shown), further demonstrating that they are different bacterial strains.

Antimicrobial assays were performed with extracts of six dominant Red Sea stony corals and six dominant soft corals against the four bacterial strains isolated from the seawater surrounding the corals. The data revealed considerable variability in natural (whole-tissue) extract concentrations and in antimicrobial activity (Tables 2, 3). Five out of six (83%) of the soft coral species inhibited at least 50% of the test bacteria, while none of the six stony coral species inhibited at least 50% of the test bacteria. From the active soft coral species examined, *X. macrospiculata* exhibited the highest antimicrobial activity (Table 3).

Bioassay-directed fractionation of the crude extract of *X. macrospiculata* indicated that the antimicrobial activity was due to the presence of a range of compounds of different polarities. One of these antibiotic compounds was isolated and identified as desoxyhavannahine (Fig. 1) by NMR spectroscopy and compared with data reported in the literature (Almourabit et al. 1989; König et al. 1989). The metabolite gave the characteristic <sup>1</sup>H-NMR signals (500 MHz, C<sub>6</sub>D<sub>6</sub>) of desoxyhavannahine at δ<sub>H</sub> 6.61 (H-1), 6.57 (H-3), 6.13 (H-13), 5.72 (H-12), 5.37 (H-14), 5.02 (H-19), 4.76 (H-19), 3.3 (H-4a), 2.9 (H-10), 2.84 (H-11a), 2.65 (H-8), 2.61 (H-18), 2.07 (H-18), 1.75 (H-17), 1.7 (Ac), 1.6 (Ac), and 1.55 (H-16) ppm; and <sup>13</sup>C-NMR signals at δ<sub>C</sub> 169.9 (s, Ac), 169.9 (s, Ac), 169.4 (s, Ac), 141.9 (s, C-11), 140.7 (d, C-3), 140.1 (s, C-15), 119.3 (d, C-14), 114.4 (t, C-19), 110.8 (s, C-4), 91.4 (d, C-1), 74.4 (d, C-12), 70.1 (d, C-13), 57.6 (d, C-8), 56.1 (d, C-9), 53.4 (s, C-7), 50.6 (t, C-18), 39.6 (d,

C-11a), 33.5 (t, C-10), 29.6 (d, C-4a), 27.3 (t, C-5), 26.1 (t, C-6), 25.5 (q, C-16), 21.1 (q, Ac), 21.1 (q, Ac), 21.0 (q, Ac), and 18.5 (q, C-17) ppm.

The estimated volumetric concentration of desoxyhavannahine in tissues of *X. macrospiculata* was ca. 590 µg cm<sup>-3</sup> (assuming 100% recovery). The MIC of purified desoxyhavannahine was 48 µg ml<sup>-1</sup> against the marine bacterium ST-1, while the MIC of the crude extract of *X. macrospiculata* was 25 µg ml<sup>-1</sup>.

## Discussion

The overall objective of the current study was to compare the ability of organic extracts of Red Sea stony corals versus soft corals collected from the same habitat, to inhibit the growth of co-occurring marine bacteria. Our results clearly showed (Tables 2, 3) that while the majority (83%) of Red Sea alcyonacean soft corals exhibited appreciable antimicrobial activity against marine bacteria isolated from the seawater surrounding the corals, the stony corals had little or no antimicrobial activity (*P* < 0.01). This lead us to conclude that these taxonomically different groups of corals may have developed different means to combat co-occurring microorganisms. While alcyonacean soft corals use chemical defense through the production of antibiotic compounds to combat microbial attack, stony corals seem to rely on other means. The usage of antibiotic disc susceptibility tests or disc-diffusion assays has the ability to rapidly identify active metabolites and therefore is particularly useful in the initial screening for antimicrobial activity and as the means for following activity during chemical purification (Jenkins et al. 1998). However, since this assay measures toxicity (cell death or inhibition of cell growth), the absence of antimicrobial activity in laboratory assays does not necessarily indicate a lack of antimicrobial chemical defense. Chemicals produced by higher organisms against co-occurring microorganisms may not simply kill or inhibit the growth of the target microorganism, but can act selectively against particular phenotypes or characteristics that are expressed by the bacteria. Maximilien et al.

**Table 2** Antimicrobial activities of extracts of Red Sea stony corals

Coral species	Extract concentration (mg cm <sup>-3</sup> of tissue ± SD)	Antimicrobial activity (mm ± SD)			
		RSW-2	RSW-3	RSW-17	RSW-18
<i>Acropora variabilis</i>	16.7 ± 4.7	0	0	0	0
<i>Fungia scutaria</i>	35.0 ± 25.0	0.3 ± 0.4	0	0.3 ± 0.4	1.0 ± 1.0
<i>Fungia granulosa</i>	23.3 ± 4.7	0	0	0	0.7 ± 0.5
<i>Turbinaria</i> sp.	20.0 ± 8.2	0	0	0	0.2 ± 0.4
<i>Stylophora pistillata</i>	33.3 ± 4.7	0	0	0	0
<i>Favia fava</i>	40.0 ± 8.2	0	0	0	0

Mean extract concentrations are expressed as mg crude extract cm<sup>-3</sup> of coral tissue ± standard deviation (SD). The amount of extract applied to each disc was equivalent to that found in 30 µl (the estimated volume of the disc) of coral tissue. Activities were tested against the Red Sea seawater bacterial strains RSW-2, RSW-3, RSW-17, and RSW-18, and are expressed as mean diameter of inhibition zone in mm ± SD

**Table 3** Antimicrobial activities of extracts of Red Sea soft corals

Coral species	Extract concentration (mg cm <sup>-3</sup> )	Extract applied (mg)	Zone of inhibition (mm)			
			RSW-2	RSW-3	RSW-17	RSW-18
<i>Litophyton arboreum</i>	47.7	1.4	1	0	0	0
	100.4	3.0	0.5	4.5	0	0
	56.8	1.7	1.5	5	0	0
Mean	68.3		1.0	3.2	0	0
SD	23.0		0.6	2.3	0	0
<i>Rythisma f. fulvum</i> <sup>a</sup>	65.8	2.0	0	1.5	0	1.5
	111.5	3.3	1.5	1.5	0	1.5
	51.2	1.5	2	1.5	0	1
Mean	76.1		1.2	1.5	0	1.3
SD	25.7		0.9	0.5	0	0.5
<i>Heteroxenia fuscescens</i>	265.2	8.0	2.5	5	0	0
	103.9	3.1	1	0	0	1
	49.6	1.5	1	2.5	0	0
Mean	139.5		1.5	2.5	0	0.3
SD	91.6		1.1	2.1	0	0.5
<i>Sarcophyton glaucum</i>	85.3	2.6	1	3.5	0	0
	481.0	14.4	0.5	5.5	0	1
	70.3	2.1	0	1	0	n.d. <sup>b</sup>
Mean	212.2		0.5	3.3	0	0.5
SD	190.2		0.5	1.9	0	0.5
<i>Dendronephthia hemprichi</i>	50.4	1.5	0	0	0	0
	10.0	0.3	0	0	0	0
	28.1	0.8	0	0	0	0
Mean	29.5		0	0	0	0
SD	16.5		0	0	0	0
<i>Xenia macrospiculata</i>	49.4	1.5	0.5	13	0	0
	54.8	1.6	7	6.5	0	0
	21.6	0.6	8	9.5	0	0
Mean	41.9		4.6	9.7	0	0
SD	14.5		3.4	2.8	0	0

Extract concentrations are expressed as mg crude extract cm<sup>-3</sup> of coral tissue. The amount of extract applied on the assay discs is expressed in mg. The amount of extract applied to each disc was equivalent to that found in 30 µl (the estimated volume of the disc) of coral tissue. Activities were tested against the Red Sea seawater bacterial strains RSW-2, RSW-3, RSW-17, and RSW-18, and are expressed as mean diameter of inhibition zone in millimeter for two replicate discs. The results are given for every coral extract, as well as the mean and SD for every coral species

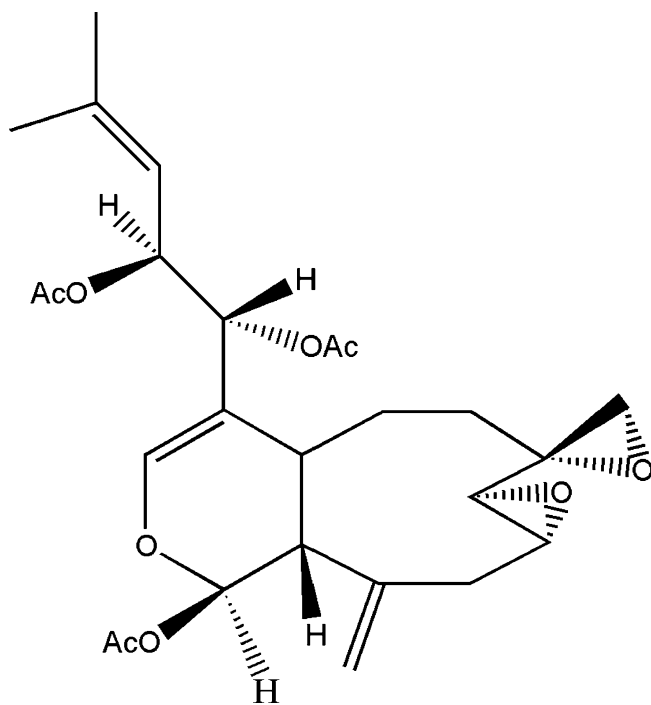
<sup>a</sup>Previously known as *Parerythropodium f. fulvum* (Alderslade 2000)

<sup>b</sup>Not determined

(1998) showed the effects of halogenated furanones from the Australian red alga *Delisea pulchra* on the colonization phenotypes of co-occurring marine bacteria. These specific phenotypes represent different stages of the bacterial fouling process that includes swimming, chemotaxis, attachment, growth, swarming, or even interference with bacterial signaling systems such as acyl homoserine lactone (AHL) regulatory system as was shown for the macroalga *D. pulchra* (Givskov et al. 1996; Rasmussen et al. 2000). Therefore, the stony corals investigated in the current study may produce metabolites that target these bacterial phenotypes rather than being toxic. Recently, AHL signal production was found in bacteria associated with marine sponges (Taylor et al. 2004). It was hypothesized that such quorum sensing signals may play a role in the chemical interactions between sponges and associated bacteria. Further work is still required to investigate the involvement of AHL inhibitory compounds in antimicrobial chemical defense. It is also possible that stony corals may produce or release antimicrobial compounds only following induction by certain deleterious microorganisms or upon

mechanical stress, as would occur if a coral was bitten by a predator. This hypothesis was not tested in the current study. However, Geffen and Rosenberg (2005) showed that the coral *Pocillopora damicornis* rapidly release antibacterials following a mechanical stress. Furthermore, in the current study we tested the activity of the coral organic extracts. It may be possible that water-soluble metabolites of stony corals may possess antimicrobial activity, as was shown by Geffen and Rosenberg (2005). On the other hand, stony corals, as opposed to soft corals, may use non-chemical defenses against microorganisms that may include mucus production and sloughing (Ducklow and Mitchell 1979b; Rublee et al. 1980).

The level of activity that is measured in the disc diffusion assay is dependent on both the rate of diffusion of the extract into the agar and the potency of the extract. Extracts that contain highly active compounds (i.e., more potent), but have physical properties that generate a lower diffusion rate, may appear to have low activity in the assay. This problem can be overcome by performing MIC assays in liquid media, as was shown for the cat-



**Fig. 1** Chemical structure of desoxyhavannahine, the antimicrobial compound present in the tissues of *X. macrospiculata*

ionic high molecular weight toxic antibiotic halitoxin (Kelman et al. 2001). The concentration of a potent compound in the crude extract is also a major factor in the activity score that is observed in laboratory assays. Extracts with a low natural (whole-tissue) concentration that exhibits high activity in laboratory assays indicates the presence of a highly potent compound. The corals that were used in the current study varied in their extract concentration (Tables 2, 3) and were not consistent with their antimicrobial activity. For example, *Sarcophyton glaucum* had a high mean extract concentration of  $212.2 \text{ mg cm}^{-3}$  but showed a much lower activity than *X. macrospiculata* that had a lower mean extract concentration of  $41.9 \text{ mg cm}^{-3}$ . These results indicate that the extracts of these corals differ in their chemical composition as well as the potency of the active metabolites.

The observed high potency of *X. macrospiculata* (Table 3) led us to choose this coral for further purification. Bioassay-directed fractionation resulted in the isolation of an active antibiotic, desoxyhavannahine (Fig. 1). However, the MIC of this compound was  $48 \text{ } \mu\text{g ml}^{-1}$ , which is about tenfold lower than its estimated natural concentration, but the MIC of the crude extract of *X. macrospiculata* was even lower ( $25 \text{ } \mu\text{g ml}^{-1}$ ). This may suggest that the extract of this coral contains additional antimicrobial compounds. This conclusion confirms what was also apparent during the fractionation process. However, due to the low concentration of these compounds, they were difficult to purify. Further work is still required in order to determine the nature of these compounds, and

to show whether these metabolites act in an additive or a synergistic fashion towards potentially harmful bacteria.

Certain symbiotic marine bacteria were shown to be responsible for the production of natural products that were previously thought to be derived from their host (Elyakov et al. 1991; Mikki et al. 1996). It is interesting to note that the soft corals that were examined in the current study were all, except *Dendronephthia hemprichi*, active against the test bacteria. *D. hemprichi* differs from the other five soft corals by the lack of symbiotic relationship with the dinoflagellate zooxanthellae. It therefore will be interesting to investigate the role of symbiotic zooxanthellae, as well as associated bacteria, in the production of natural products, especially metabolites that target co-occurring and potentially harmful microorganisms.

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