



Antifungal properties of Singapore gorgonians: a preliminary study

L.L. Koh^{*}, T.K. Tan, L.M. Chou, N.K.C. Goh

*Department of Biological Sciences, National University of Singapore, Blk. S2, 14 Science Drive 4,
Singapore 117543, Singapore*

Abstract

Gorgonians possess a huge array of secondary metabolites for various functions, many of which are not known. One of these functions is antifungal. This study investigates if gorgonians from reefs in Singapore can defend themselves against the settlement and invasion of fungi. Crude extracts from 10 species of gorgonians from three families, Ellisellidae, Subergorgiidae and Plexauridae, were screened against nine species of deuteromycetous fungi previously isolated from gorgonians. Minimum Inhibitory Concentration (MIC) experiments were carried out in 96-microwell plates using extract concentrations ranging from 1.5 to 24.0 mg/ml. Extracts from *Euplexaura* cf. *pinnata*, *Echinogorgia* sp. C, *Junceella* cf. *gemmacea*, *Subergorgia suberosa*, *Ctenocella* cf. *umbraculum* and *Junceella* sp. A were found to possess inhibitory effects on fungi. MICs range from 1.5 to 18.0 mg/ml. Results showed that most of the antifungal activities were exhibited by *Euplexaura* cf. *pinnata* and *Echinogorgia* sp. C from the family Plexauridae. However, for most gorgonian species, the concentrations required to inhibit fungal growth are much higher than the natural concentrations of extracts found in gorgonian tissues. Only the extracts of *Echinogorgia* sp. C, C. cf. *umbraculum* and *S. suberosa* were found to inhibit fungal growth of a few fungal species at a concentration lower than that of its natural concentration. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Gorgonians; Deuteromycetous fungi; Antifungal; Secondary metabolites; Minimum Inhibitory Concentration

1. Introduction

Gorgonians and other octocorals are known to possess a huge array of secondary metabolites, which play important roles in protecting the colonies against grazing and

^{*} Corresponding author. Tel.: +65-778-7112; fax: +65-779-2486.

E-mail addresses: scip9543@nus.edu.sg, liling_koh@hotmail.com (L.L. Koh).

feeding (Gerhart, 1986; Harvell and Fenical, 1989; Paul, 1992; Pawlik, 1993; Bolser and Hay, 1996; Koh et al., 2000). Terpenoid secondary metabolites present in certain species of octocorals were known to cause tissue necrosis upon contact with neighbouring corals or through the water column in the absence of contact, achieving an allelopathic function (Coll et al., 1982; Sammarco and Coll, 1992). A range of cembranoid diterpenes from octocorals (Coll et al., 1987) as well as crude extracts of gorgonians (Wilsanand et al., 1999, 2001) were also known to have antifouling properties. These secondary metabolites produced by gorgonians can inhibit the settlement of algae and both the adult and larval form of marine organisms such as barnacles. Lastly, compounds from the non-polar fractions of gorgonian crude extracts were known to mediate coral-microbe (Kim, 1994). Antimicrobial activities of gorgonians have also been studied by several researchers (Kim, 1994; Goh et al., 1995; Jensen et al., 1996). Marine organisms possess a glycoproteinaeous film which favours colonisation by bacteria, fungi, diatoms, protozoa and other microorganisms (Hellio et al., 2000). These microbes may be pathogenic or may cause fouling on the surfaces of hosts (Kim, 1994).

Gorgonians, being sessile organisms are susceptible to invasion by microbial pathogens such as bacteria and fungi, hence the need to possess defenses against such pathogens. Other sessile organisms such as holothurians, sea urchins and hard corals are also susceptible to microbial invasion. Although Holothurians are known to contain triterpene glycosides, which show antifungal activity, fungi can still be isolated from these organisms. *Cladosporium brevicompactum* and *C. sphaerospermum* are common in the holothurians' coelom. *Cladosporium* spp., *Aspergillus* spp. and *Penicillium* spp. were found on the body surface of the holothurians (Pivkin, 2000). Fungi were also found to damage spines of sea urchins, *Diadema antillarum* (Mortensen, 1940). Fungi associations with hard corals are better known and are usually manifested in the form of diseases. Twelve genera of deuteromycetous fungi have been isolated from hard corals and hydrocorals (Kendrick et al., 1982). Bak and Lanne (1987) observed black bands made up of high concentrations of a dark fungus in *Porites*. The fungus, *Scolecobasidium* sp. was found to cause necrotic patches on corals from the Andaman Islands (Raghukumar and Raghukumar, 1991). Le Campion-Alsumard et al. (1995) have also found fungi to bore through the skeleton of live *Porites*. Fungi are able to bore through the skeleton of hard corals by chemical dissolution and they are also able to utilize the organic matrix of coral skeletons (Glynn, 1997).

Recently, widespread epizootic disease in gorgonians has been reported in the Caribbean and this disease is known to be caused by *Aspergillus sydowii* (Smith et al., 1996; Geiser et al., 1998). The question which then follows is, if gorgonians possess chemical defenses against pathogens, then why is *A. sydowii* still able to invade the tissues? There are several postulations: (1) epizootic diseases are facilitated by environmental stressors such as elevated water temperature which compromise resistance in corals (Le Campion-Alsumard et al., 1995; Cerrano et al., 2000; Kim et al., 2000b); (2) *Aspergillus* spp. are very opportunistic and known to attack immune-compromised hosts (Geiser et al., 1998); (3) certain fungi may have adapted and developed resistance against fungicidal compounds in host (Pivkin, 2000). In other words, diseases occur as a result of interactions between a susceptible host, a virulent pathogen and prevailing environmental conditions (Peters, 1997). There is still very little known about the mechanism of

gorgonians' resistance to diseases. Chemical defense may just be one of the many. There had been several attempts to investigate the mechanism of defense employed by gorgonians against fungal infection. Kim et al. (2000a) tested the crude extracts of *Gorgia ventalina* and *G. flabellum* against *A. sydowii* and found the crude extracts of both gorgonians to be effective against the fungus at concentrations of 5–10 mg/ml. Kim et al. (2000b) tested the crude extracts of another 20 common gorgonians species in the Florida Keys against *A. sydowii*. Findings showed that several species of gorgonians from the genera *Pseudoplexaura* and *Psuedopterogorgia* were most active against the fungus. Slattery (1999) also investigated the effect of *A. sydowii* on gorgonian chemical and structural defense as well as the potential for local adaptation of the fungal pathogen to its seafan host. Results showed that the tested gorgonian, *G. ventalina*, responded both physically and chemically against the fungal infection. *A. sydowii* was also found to be most virulent at its original site of occurrence. The above studies indicate that gorgonians in the Caribbean are able to defend themselves against fungal invasion.

Gorgonians on Singapore's reefs are constantly subjected to environmental stressors such as heavy sedimentation. Such stressors compromise the host's immune system, making it easy for fungi to invade the hosts' tissues. This study aims to investigate if gorgonians in Singapore possess any compound to protect themselves against fungal invasion.

2. Materials and methods

2.1. Sample collection

Gorgonians used in this study were collected from Raffles Lighthouse (1°09' 5"N, 103°44' 5"E) by SCUBA, a fringing reef south of Singapore. Samples were stored in ice immediately after collection and eventually stored at –20°C in the laboratory freezer. Ten species of gorgonians from three families were used for this study: from the family Ellisellidae: *Junceella* sp. A (as described in Goh and Chou, 1996), *Junceella* (*Dichotella*) cf. *gemmacea* (Valenciennes), *Ctenocella* (*Umbracella*) sp. A (as described in Goh and Chou, 1996) and *Ctenocella* cf. *umbraculum* (Ellis and Solander); from the family Subergorgiidae: *Subergorgia mollis* (Nutting) and *S. suberosa* (Pallas); from the family Plexauridae: *Echinogorgia* sp. A (as described in Goh and Chou, 1996), *Echinogorgia* sp. C (as described in Goh and Chou, 1996), *Echinogorgia* sp. E (as described in Goh and Chou, 1996) and *Euplexaura* cf. *pinnata* (Nutting)

2.2. Extraction

Frozen samples were cut into small pieces and weighed. The tissues were then immersed in a known volume of dichloromethane and left overnight. The volume of gorgonians used was calculated by simple displacement. This solvent extraction was repeated two times with fresh solvent. The solvents from the three extractions were combined and evaporated to yield the crude extract. Natural concentration was calculated by dividing the weight of the extract by the volume of gorgonian extracted (obtained by

displacement). However, extracts in gorgonians are usually concentrated in the coenenchyme and not the axes (West, 1998). To obtain a better estimation of the natural concentration of extracts in gorgonians, another calculation was done by excluding the volume of axes from the total volume of gorgonians. The volume of gorgonian axes was calculated and expressed as a percentage of the total volume of gorgonians. Volume of axes was measured using the following formulas:

$$\text{Volume of gorgonian axis (A)} = \pi (\text{Diameter of axis}/2)^2 \times \text{Length}$$

$$\begin{aligned} \text{Volume of gorgonian axis + coenenchyme (B)} \\ = \pi (\text{Diameter of axis + coenenchyme}/2)^2 \times \text{Length} \end{aligned}$$

$$\text{Percentage of axes over total volume of gorgonians} = A/B \times 100\%$$

2.3. Antifungal assays

The protocol employed here follows that of Kim et al. (2000a) closely. Crude extracts of the 10 species of gorgonians were tested against nine species of ubiquitous fungi previously isolated from gorgonians (Koh et al., in press). The nine species of fungi used were: *A. flavus* Link, *Trichoderma pseudokoningii* Rifai, *C. sphaerospermum* Penz, *Gliomastix cerealis* (Karst) Dickinson, *Acremonium furcatum* F. and V. Moreau ex. W. Gams, *Tritirachium* sp., *Scolecobasidium humicola* Barron and Busch, *Penicillium citrinum* and *A. foetidus* var. *pallidus* Naka, Simo and Wat.

The experiment was carried out in 96-microwell plates. To each well, 75 μl of sterile fungal broth, 25 μl of spore suspension containing approximately 2.5×10^4 to 3.0×10^4 spores and crude extracts were added. The crude extracts were weighed and reconstituted in acetone. Concentrations of extract used were 1.5, 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0 and 24.0 mg/ml. Two controls were set up for each experiment: one consisted of fungal broth and fungal spores only, the other consisted of acetone, fungal broth and fungal spores. The first control aimed to show viability of spores while the second control aimed to show the effect of using acetone as a carrier for the crude extracts.

Antifungal properties of the crude extracts were tested against the various fungal spores using Minimum Inhibitory Concentrations (MIC). As the mixture in each well was too cloudy to observe any hyphal growth by direct microscope, 15 μl of the mixture from each well was plated onto Potato Dextrose Agar (PDA). This was done after 5 days of incubation or when hyphal growth was observed in the clear control wells. These plates were then incubated at 27 °C. Contents from wells in which fungal growth was inhibited would not show any fungal growth on PDA while those in which there was no inhibition would show fungal growth on PDA. The lowest concentration at which fungal growth was inhibited was taken as the MIC of the particular gorgonian species. The crude extracts were first screened for antifungal properties. Any antifungal properties shown were confirmed with three additional replicates to ensure that results obtained were accurate.

3. Results

The natural concentration of extracts obtained from each species of gorgonians is shown in Table 1. It ranges from 2.78 mg/ml for *Junceella* cf. *gemmacea* to 21.2 mg/ml for *S. mollis*. With a few exceptions, there was a general trend that gorgonians with reticulate morphology such as *S. mollis* have higher extract concentrations than gorgonians which are whip-like (*Junceella* sp. A) or dichotomously branched (*J. cf. gemmacea*). However, it is known that extracts in gorgonians are usually concentrated in the coenenchyme and not the axes. To obtain a better estimation of the natural concentration of extracts in gorgonians, another calculation was done by excluding the volume of axes from the total volume of gorgonians (Table 1). Axis volume ranges from 12.75% to 60.76% of the total gorgonian volume. Natural concentration of extracts now ranges from 4.34 mg/ml for *Junceella* cf. *gemmacea* to 54.05 mg/ml for *S. mollis*.

Table 2 shows the effects of gorgonian extracts on the nine species of fungi. It was found that *S. mollis*, *Ctenocella* (*Umbracella*) sp. A, *Echinogorgia* sp. A and *Echinogorgia* sp. E were not effective against any of the nine species of fungi at the tested concentrations of 1.5 to 24.0 mg/ml. However, *S. mollis* had a high natural concentration of 54.05 mg/ml but there was no testing at this concentration. The rest of the gorgonians showed inhibition against certain species of fungi with MIC ranging from 1.5 to 18.0 mg/ml. *Junceella* sp. A was inhibited at 9 mg/ml, *C. cf. umbraculum* at 12 mg/ml, *S. suberosa* at 12 mg/ml *Echinogorgia* sp. C at 3 mg/ml and *Euplexaura* cf. *pinnata* at 13.5 mg/ml. *Tritirachium* sp. appeared to be the most susceptible with extracts of five gorgonian species inhibiting its growth. *T. pseudokoningii* was inhibited by *J. cf. gemmacea*, *Echinogorgia* sp. C and *Euplexaura* cf. *pinnata* at concentrations of 13, 9 and 14 mg/ml, respectively. *A. foetidus* var. *pallidus* was inhibited by *Echinogorgia* sp. C only at a concentration of 18 mg/ml. *C. sphaerospermum*, *G. cerealis* and *A. furcatum* were inhibited by *Euplexaura* cf. *pinnata* at 15, 13 and 18 mg/ml, respectively. The results showed that some gorgonians have extracts that could be deterrent against fungal growth but mostly at concentrations much higher than the calculated natural concentrations. Only

Table 1
Natural concentrations of extracts obtained from gorgonians

Species of gorgonians	Concentration of extract (mg/ml)	Percentage of axes over total volume of gorgonians (%)	Concentration of extract excluding volume of axes (mg/ml)
<i>Subergorgia mollis</i>	21.20	60.76	54.05
<i>Ctenocella</i> cf. <i>umbraculum</i>	14.75	29.60	20.96
<i>Echinogorgia</i> sp. A	12.48	18.75	15.37
<i>Echinogorgia</i> sp. E	12.40	28.23	17.26
<i>Ctenocella</i> (<i>Umbracella</i>) sp. A	10.90	26.44	14.83
<i>Subergorgia suberosa</i>	9.31	29.27	13.14
<i>Echinogorgia</i> sp. C	7.53	12.75	8.63
<i>Junceella</i> sp. A	4.63	26.50	6.07
<i>Euplexaura</i> cf. <i>pinnata</i>	4.14	32.97	6.19
<i>Junceella</i> (<i>Dichotella</i>) cf. <i>gemmacea</i>	2.78	32.15	4.34

Table 2

Effects of gorgonian extracts with concentrations ranging from 1.5 to 24 mg/ml on nine species of fungi

Species of gorgonians	1	2	3	4	5	6	7	8	9
<i>Subergorgia mollis</i>	–	–	–	–	–	–	–	–	–
<i>Ctenocella (Umbracella)</i> sp. A	–	–	–	–	–	–	–	–	–
<i>Echinogorgia</i> sp. A	–	–	–	–	–	–	–	–	–
<i>Echinogorgia</i> sp. E	–	–	–	–	–	–	–	–	–
<i>Junceella</i> sp. A	–	–	–	–	–	–	+9 (6.07)	–	–
<i>Ctenocella</i> cf. <i>umbraculum</i>	–	–	–	–	–	–	+12* (20.96)	–	–
<i>Subergorgia suberosa</i>	–	–	–	–	–	–	+12* (13.14)	–	–
<i>Junceella (Dichotella)</i> cf. <i>gemmacea</i>	–	+13 (4.34)	–	–	–	–	–	–	–
<i>Echinogorgia</i> sp. C	+18 (8.63)	+9 (8.63)	–	–	–	–	+3* (8.63)	–	+1.5* (8.63)
<i>Euplexaura</i> cf. <i>pinnata</i>	–	+14 (6.19)	+15 (6.19)	–	+13 (6.19)	+18 (6.19)	+13.5 (6.19)	–	–

Key: 1: *A. foetidus* var. *pallidus*, 2: *T. pseudokoningii*, 3: *C. sphaerospermum*, 4: *P. citrinum* (C), 5: *G. cerealis*, 6: *A. furcatum*, 7: *Tritirachium* sp., 8: *A. flavus* Link, 9: *S. humicola*.

(–) Denotes that extract does not inhibit fungal growth; (+) denotes that extract inhibited fungal growth. The numbers show the Minimum Inhibitory Concentration (mg/ml). Numbers in parentheses denote natural concentration. (*) Denotes that gorgonian extracts are effective against fungal growth at or below their natural concentrations.

C. cf. umbraculum, *S. suberosa* and *Echinogorgia* sp. C yielded extracts effective against certain fungal species at their natural calculated concentrations. *Ctenocella. cf. umbraculum* was effective against *Tritirachium* sp. at 12 mg/ml which was also below its calculated natural concentration of 20.96 mg/ml. *S. suberosa* was effective against *Tritirachium* sp. at 12 mg/ml which was also below its natural concentration of 13.14 mg/ml. *Echinogorgia* sp. C was effective against *Tritirachium* sp. and *S. humicola* at 3 and 1.5 mg/ml, respectively, while its calculated natural concentration was 8.63 mg/ml. *P. citrinum* and *A. flavus* Link were the most resistant against gorgonian extracts. None of the extract was found to be effective against these two fungal species. *Euplexaura* cf. *pinnata* was the most effective against fungi, inhibiting the growth of five out of the nine tested fungal species, but at concentrations higher than its natural concentration.

4. Discussion

Minimum Inhibitory Concentration assays were employed in this experiment as they are commonly used to investigate the efficacy of drugs against filamentous fungi (Kim et al., 2000a). This method is more superior than disk diffusion assays which were first developed for bacteria (De Beer and Sherwood, 1945) and subsequently used by many other researchers for the study of antimicrobial activities (Kim, 1994; Goh et al., 1995; Jensen et al., 1996). In disk diffusion assays, the potencies of extracts are measured by the

diameter of the zone of inhibition. The wider the zone of inhibition, the more toxic an extract is. MIC assays have the advantage of providing the absolute value or concentration at which an extract is effective against the test microorganism. Another advantage of MIC method is the homogeneity of the experiment. Fungal spores are uniformly bathed in the fungal broth and gorgonian extracts, giving more accurate results. For disk diffusion assays, the amount of test compound which diffuses into the agar medium is not uniform, making it difficult to quantify MIC values. However, the definition of MIC value is not always clear. In diffusion assays, MIC is defined as the lowest concentration of test compound producing a visible inhibition effect. While in dilution assays, MIC is defined as the lowest concentration of test compound totally inhibiting growth. These two values are actually not comparable (Hadacek and Greger, 2000). In this study, MIC is defined as the lowest concentration of test compound, which inhibits fungal growth totally. Also, for the comparison of bioactivity of compounds in this study, MIC assays are more suitable. Disk diffusion assays are also more suitable for polar than non-polar substances (Janssen et al., 1987). In this case, we are dealing with non-polar substances.

This is one of the first attempts to investigate the antifungal properties of gorgonians in Southeast Asia. Most of such work had previously been done by Kim et al. (2000a) in the Caribbean on *G. ventalina* and *G. flabellum*. These two species of gorgonians are not found in Singapore, instead 10 other species of gorgonians were chosen for this study. The chosen gorgonians represent the more common species on the reefs in Singapore. It is important to choose common species for this study as sizes of gorgonian colonies are severely limited by heavy sedimentation in water. Common species will ensure that sufficient colonies are collected for extraction purposes. These species were previously investigated for their chemical and physical defenses against fish predation (Koh et al., 2000). This will allow us to make a correlation between antifungal and antifeeding properties of gorgonians.

In this study, it was shown that *S. mollis* has the highest average natural extract concentration in its tissue but was not effective against any of the fungal species tested even at a concentration of 24.0 mg/ml (Tables 1 and 2). *Ctenocella (Umbracella)* sp. A and *Echinogorgia* sp. E did not inhibit fungal growth the highest tested extract concentration as well. This shows that the amount of extract present in the tissues of gorgonians bore no relationship with its antifungal activity. There are several possible factors influencing the above phenomenon. A possible factor is the distribution of extract in the tissues. The value of natural concentration depends on whether the extract is distributed only on the colony surface or throughout the tissues, and whether the extract is more concentrated at the colony edge or distributed evenly throughout the entire colony (Kim et al., 2000b). Taking this factor into consideration, volume of gorgonian axis was excluded from the total volume in the calculation of natural concentration. This gave a better estimate of the natural concentrations of gorgonian extracts. However, species with high extract concentration such as *S. mollis* (54.05 mg/ml) still did not deter fungal growth while *Junceella* cf. *gemmacea* with the lowest extract concentration (4.34 mg/ml) deterred fungal growth. This suggests that although extract concentration in gorgonian tissues may be high, the percentage of bioactive compounds in the extract may be low, hence the lack of antifungal activity.

The results showed that most of the MICs were much higher than the natural concentrations of extracts in gorgonians. It is possible that only in times when fungi

manifested themselves as pathogens, that the gorgonian would increase the extract concentrations around the diseased tissues. Slattery (1999) showed that although there was little change in the crude extract concentrations near the site of tissue necrosis, measurable changes were observed at sites away from the lesions in *G. ventalina*. This shows that fungal pathogenesis actually induces chemical responses from gorgonians. This phenomenon is more well-documented in algae. Paul and Van Alstyne (1992) showed that weakly deterrent metabolites in the algae were converted to more strongly deterrent metabolites if the algal tissues were damaged. This process was termed as 'activation'.

Species of *Trichoderma*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Tritirachium*, *Scolecobasidium*, *Gliomatix* and *Acremonium* were chosen for this experiment because they were commonly isolated from gorgonians in Singapore (Koh et al., in press). They were isolated from both healthy and unhealthy gorgonians. Although these fungi were almost always found growing on these gorgonians, they were not noticed to cause any signs of diseases. It led us to suspect that fungi are part of the natural mycoflora of gorgonians and they possess the ability to keep these fungi in check. It is however not known if these fungi benefit from gorgonians or harm them in anyway. The fungi were not observed to have any specific trend of association with gorgonians (Koh et al., in press). Although gorgonians exhibit inter-species and intra-species variations in the secondary metabolites they possess (Puglisi et al., 2000), no study has been done to show that such variations influence the species of fungi with gorgonians. In the Ligurian sea, *Trichoderma* sp., *Cladosporium* sp. and *Penicillium* sp. were also found growing on damaged gorgonian tissues (Cerrano et al., 2000). These fungi may be just opportunistic invaders and not the primary pathogens. Usually, gorgonians possess the ability to keep these fungi under control but in times of environmental stress, these fungi manifest themselves as diseases (Goreau et al., 1998).

An attempt was made to correlate predator deterrence of gorgonians with their antifungal properties. It was shown that gorgonian extracts from the family Ellisellidae were more deterrent towards fishes than extracts from the family Plexauridae (Koh et al., 2000). However, the findings of this study showed that *Euplexaura* cf. *pinnata* from the family Plexauridae inhibited the growth of five fungal species while *Echinogorgia* sp. C which is also from the family Plexauridae inhibited the two fungal species. In comparison, gorgonian species from the family Ellisellidae inhibit the growth of only one or two fungal species. Hence, it can be concluded that the effectiveness of gorgonian extracts against predators cannot reflect their antifungal properties. Slattery (1999) postulated that the production of a feeding deterrent compound decreases following a fungal infection. This is because the costly process of producing a feeding deterrent compound must be balanced by tissue repair. Hence, we can deduce that a gorgonian species, which has extracts effective against fungi, will not be as effective against predators and vice versa.

It is important for future research to concentrate on finding the mechanisms employed by gorgonians to defend themselves against fungal invasion, the mechanism of fungal infection and the type of chemical compound in gorgonian extracts that inhibit fungal growth or proliferation. Another aspect to look into is the differences in the extract concentrations between healthy and unhealthy gorgonians. This will give a better idea of how gorgonians respond to fungal infection. It will also be interesting to find out if the MICs of gorgonian extracts against fungi differ between healthy and unhealthy gorgonians.

Acknowledgements

We would like to thank H.H. Tan, Tommy Tan, C.H. Lai and Sharon Lee for assisting in field work. We acknowledge National University of Singapore for the provision of a research scholarship. [SS]

References

- Bak, R.P.M., Lanne, R.W.P.M., 1987. Annual black bands in skeletons of reef corals (Scleractinia). *Mar. Ecol., Prog. Ser.* 38, 169–175.
- Bolser, R.C., Hay, M.E., 1996. Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. *Ecology* 77 (8), 2269–2286.
- Cerrano, C., Bavestrello, G., Bianchi, N., Cattano-Vietti, R., Bava, S., Morganti, C., Morri, C., Picco, P., Sara, G., Schiaparelli, S., Siccardi, A., Sponga, F., 2000. A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. *Ecol. Lett.* 3, 284–293.
- Coll, J.C., Bowden, B.F., Tapiolas, D.M., Dunlap, W.C., 1982. In situ isolation of allelochemicals releases from soft corals (Coelenterata: Octocorallia): a totally submersible sampling apparatus. *J. Exp. Mar. Biol. Ecol.* 60, 293–299.
- Coll, J.C., Price, I.R., Konig, G.M., Bowden, B.F., 1987. Algal overgrowth of alcyonacean soft corals. *Mar. Biol.* 96, 129–135.
- De Beer, E.J., Sherwood, M.B., 1945. The paper-disk agar plate method for the assay of antibiotic substances. *J. Bacteriol.* 30, 459–468.
- Geiser, D.M., Taylor, J.W., Ritchie, K.B., Smith, G.W., 1998. Cause of sea fan death in the West Indies. *Nature* 394, 137–138.
- Gerhart, D.J., 1986. Gregariousness in the gorgonian-eating gastropod *Cyphoma gibbosum*: test of several causes. *Mar. Ecol., Prog. Ser.* 75, 1–8.
- Glynn, P.W., 1997. Bioerosion and coral reef growth: a dynamic balance. In: Birkeland, C. (Ed.), *Life and Death of Coral Reefs*. Chapman & Hall, New York, pp. 68–95.
- Goh, N.K.C., Chou, L.M., 1996. An annotated checklist of the gorgonians (Anthozoa: Octocorallia) of Singapore, with a discussion of gorgonian diversity on the Indo-West Pacific. *Raffles Bull. Zool.* 44 (2), 435–459.
- Goh, N.K.C., Sim, T.S., Chou, L.M., 1995. Bioactivity of gorgonians (Sub-class Octocorallia) in Singapore: preliminary studies. *Mar. Res.* 4 (1), 33–46.
- Goreau, T.J., Cervino, J., Goreau, M., Hayes, R., Hayes, M., Richardson, L., Smith, G., Demeyer, K., Nagelkerken, I., Garzon-Ferrera, J., Gil, D., Garrison, G., Williams, E.H., Bunkley-Williams, L., Quiro, C., Patterson, K., Porter, J.W., Porter, K., 1998. Rapid spread of diseases in Caribbean coral reefs. *Rev. Biol. Trop.* 46 (Suppl. 5), 157–171.
- Hadacek, F., Greger, H., 2000. Testing of antifungal natural products: methodologies, comparability of results and assay choice. *Phytochem. Anal.* 11, 137–147.
- Harvell, C.D., Fenical, W., 1989. Chemical and structural defenses of Caribbean gorgonians (*Pseudoterogorgia* spp.): intracolony localization of defense. *Limnol. Oceanogr.* 34, 382–389.
- Hellio, C., Bremer, G., Pons, A.M., Le Gal, Y., Bourgougnon, N., 2000. Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. *Appl. Microbiol. Biotechnol.* 54, 543–549.
- Janssen, M., Scheffér, J.C., Svendsen, A.B., 1987. Antimicrobial activity of essential oils: a 1976–1986 literature review. *Planta Med.* 53, 395–398.
- Jensen, P.R., Harvell, C.D., Wirtz, K., Fenical, W., 1996. Antimicrobial activity of extracts of Caribbean gorgonian corals. *Mar. Biol.* 125, 411–419.
- Kendrick, B., Risk, M.J., Michaelides, J., Bergman, K., 1982. Amphibious microborers: bioeroding fungi isolated from live corals. *Bull. Mar. Sci.* 32, 862–867.
- Kim, K., 1994. Antimicrobial activity in gorgonian corals (Coelenterata, Octocorallia). *Coral Reefs* 13, 75–80.
- Kim, K., Harvell, C.D., Kim, P.D., Smith, G.W., Merkel, S.M., 2000a. Fungal disease resistance of Caribbean sea fan corals (*Gorgonia* spp.). *Mar. Biol.* 136, 259–267.

- Kim, K., Kim, P.D., Alker, A.P., Harvell, C.D., 2000b. Chemical resistance of gorgonian corals against fungal infections. *Mar. Biol.* 137, 393–401.
- Koh, L.L., Goh, N.K.C., Chou, L.M., Tan, Y.W., 2000. Chemical and physical defenses of Singapore gorgonians (Octocorallia: Gorgonacea). *J. Exp. Mar. Biol. Ecol.* 251, 103–115.
- Koh, L.L., Tan, T.K., Chou, L.M., Goh, N.K.C., in press. Fungi associated with gorgonians in Singapore. *Proceedings of the Ninth International Coral Reefs Symposium*.
- Le Campion-Alsumard, T., Golubic, S., Priess, K., 1995. Fungi in corals: symbiosis or disease? Interactions between polyps and fungi causes pearl-like skeleton biomineralization. *Mar. Ecol., Prog. Ser.* 117, 137–147.
- Mortensen, T., 1940. A Monograp of the Echinoidea: Part III. 1 Aulodonta C.A. Reitzel, Copenhagen.
- Paul, V.J., 1992. Chemical defenses of benthic marine invertebrates. In: Paul, V.J. (Ed.), *Ecological Roles of Marine Natural Products*. Cornstock and Publishing Associates, London, pp. 164–188.
- Paul, V.J., Van Alstyne, K.L., 1992. Activation of chemical defenses in the tropical green algae *Halimeda* spp. *J. Exp. Mar. Biol. Ecol.* 160, 191–203.
- Pawlik, J.R., 1993. Marine invertebrate chemical defense. *Chem. Rev.* 93 (5), 1911–1922.
- Peters, E., 1997. Diseases of coral reef organisms. In: Birkeland, C. (Ed.), *Life and Death of Coral Reefs*. Chapman & Hall, New York, pp. 114–139.
- Pivkin, M.V., 2000. Filamentous fungi associated with holothurians from the sea of Japan, off the Primorye Coast of Russia. *Biol. Bull.* 198, 101–109.
- Puglisi, M.P., Paul, V.J., Slattery, M., 2000. Biogeographic comparisons of chemical and structural defenses of Pacific gorgonians *Annella mollis* and *A. reticulata*. *Mar. Ecol., Prog. Ser.* 207, 263–272.
- Raghukumar, C., Raghukumar, S., 1991. Fungal invasion of massive corals. *PSZNI: Mar. Ecol.* 12 (3), 251–260.
- Sammarco, P.W., Coll, J.C., 1992. Chemical adaptations in the Octocorallia: evolutionary considerations. *Mar. Ecol., Prog. Ser.* 88, 93–104.
- Slattery, M., 1999. Fungal pathogenesis of the sea fan *Gorgonia ventalina*: direct and indirect consequences. *Chemoecology* 9, 97–104.
- Smith, G.W., Ives, L.D., Nagelkerken, I.A., Ritchie, K.B., 1996. Caribbean sea-fan mortalities. *Nature* 383, 487.
- West, J.M., 1998. The dual role of sclerites in gorgonian corals: conflicting functions of support and defense. *Evol. Ecol.* 12, 803–821.
- Wilsanand, V., Wagh, A.B., Bapuji, M., 1999. Antifouling activities of marine sedentary invertebrates on some macrofoulers. *Indian J. Mar. Sci.* 28 (3), 280–284.
- Wilsanand, V., Wagh, A.B., Bapuji, M., 2001. Antifouling activities of octocorals on some marine microfoulers. *Microbios* 104 (409), 131–140.