

# Anti-Biofouling Defence Mechanism of Basibionts (A Chemical Warfare) - A Critical Review

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## Abstract

The fouling process is an ecologically complex web of interactions between basibionts e.g., corals, surface-colonizing microbes e.g., bacteria, and fouling biota e.g., *Balanus species* which are all mediated by chemical signalling. Sessile invertebrates, such as soft corals, sponges and sea cucumbers, evolved in an intense competitive milieu for space, light and nutrients, therefore they have developed chemical defence mechanism by producing secondary metabolites e.g., Terpenes to ward off bio-foulers and maintain clean body surfaces. The settlement of surface-colonizing organisms, commonly referred to as bio-fouling organisms, occurs naturally in a turbulent environment, yet the effects of waterborne versus surface-adsorbed chemical defences have not been compared in flow, therefore limiting our understanding of how they respond to toxic surfaces of the basibionts. Here, we reviewed the evidence that basibionts chemically inhibit the propagules of fouling organisms under natural conditions, and that chemosensory mechanisms may allow the larvae of bio-fouling animals to detect and avoid settling on chemically protected basibionts.

**Keywords:** Fouling biota; Basibionts; Settlement; Chemical signalling; Toxic surfaces

## Introduction

Basibionts are substrate or benthic organisms which are the hosts to epibionts or bio-foulers. Corals and sponges are the most studied groups of benthic invertebrates in marine chemical ecology due to their abundance and distribution in all seas [1,2]. Several studies have been conducted by the benthic ecologists and chemists to unravel the mechanisms of chemical defence of the basibionts which protect their surfaces against fouling from epibiotic association. Many marine invertebrates such as soft corals and sea cucumbers are sessile i.e., steadily attached to the sea bottom or with low movement, thus vulnerable either to predation and threat from a rich surrounding microbiota with pathogenic potential. One of the most important challenges for the benthic organisms is to combat the problems of biofouling. 'Biofouling' is the colonisation of submerged surfaces by unwanted organisms such as bacteria, barnacles, algae, etc and has detrimental effects on shipping and leisure vessels, heat exchangers, oceanographic sensors and mariculture, with considerable ecological and economic consequences [3]. Soft corals and sea cucumbers are under intense competitive pressure for space, light, and nutrients. Fouling can have severely deleterious effects on benthic organisms, such as inhibition of photosynthesis, blockage of filter feeding, and elevated risk of mechanical dislodgement or predation. It is not surprising that they have developed a range of chemical defences to ensure their survival. Biofouling has been shown to be a sequential process [4], one stage of succession being conducive to the onset of the next [5]. Although these mechanisms are somewhat different for micro- and macro-organisms the sequence of events follows a similar pattern (Figure 1): settlement, attachment, development and growth of foulers such as bacteria, protists, barnacles, bivalves, hydroids, sedentary polychaetes, bryozoans, anemones, tunicates, diatoms, as well as green, brown and red algae [6-10]. Fouling is described as an on-going process which has no true end, as even a mature fouling community will undergo changes in composition due to season, disturbance, predation, and other biological and abiotic influences. As said earlier, soft corals, sponges, sea cucumbers live in close association with microorganisms like bacteria and other bio-foulers like barnacles and their body surfaces are inevitably colonized by these

epibionts; while some of them harbour microorganisms within their digestive tracts or even within tissues and cells. Such interactions are complex and reach from harmful diseases to symbioses of mutual benefit [11]. Associated microorganisms have recently been shown to be involved in the synthesis of numerous metabolites [12]. Numerous studies demonstrate secondary metabolite production by symbionts such as the synthesis of the bicyclic glycopeptide theopalauamide by an associated delta-proteobacterium in the sponge, *Theonella swinhoei* [13], the synthesis of bryostatin by bacterial symbionts in the bryozoan, *Bugula neritina* [14], or the antimicrobial activity of different bacterial strains isolated from the sponges, *Aplysina aerophoba* and *A. cavernicola* [15]. Bio-foulers like some microbes play a double role in chemical interactions with higher organisms like the corals. They can be harmful and are repelled by chemical defences or they may be useful symbionts for their hosts by providing protection and camouflage against predators hunting by visual or chemical cues [16,17]. Soft corals and sea cucumbers have evolved mechanisms that enable them to distinguish between beneficial and detrimental biofoulers. Secondary metabolites act as a controlling factor in this host-biofouler interaction. They are used as a defence strategy against unwanted colonization (infection) by bio-foulers. These sessile invertebrates, soft corals, sponges, and sea cucumbers (Figure 2), produce an astonishing variety of anti-biofouling compounds (structures in Figure 3) [18], which help them to ward off surface colonization [19,20].

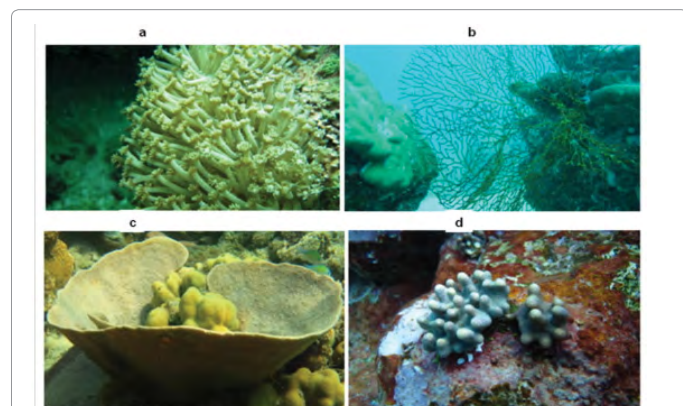
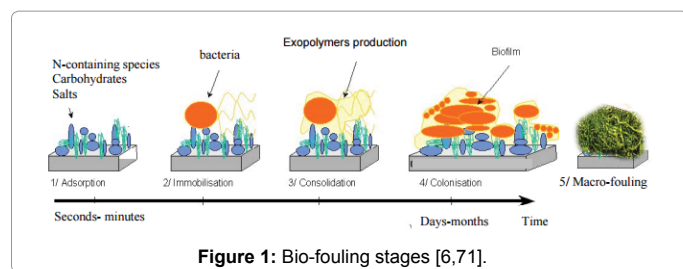
The aim and objective of this review is to focus on chemical defence mechanisms of some hard and soft corals, sponges and sea cucumbers against multiple fouling organisms or epibionts in the field.

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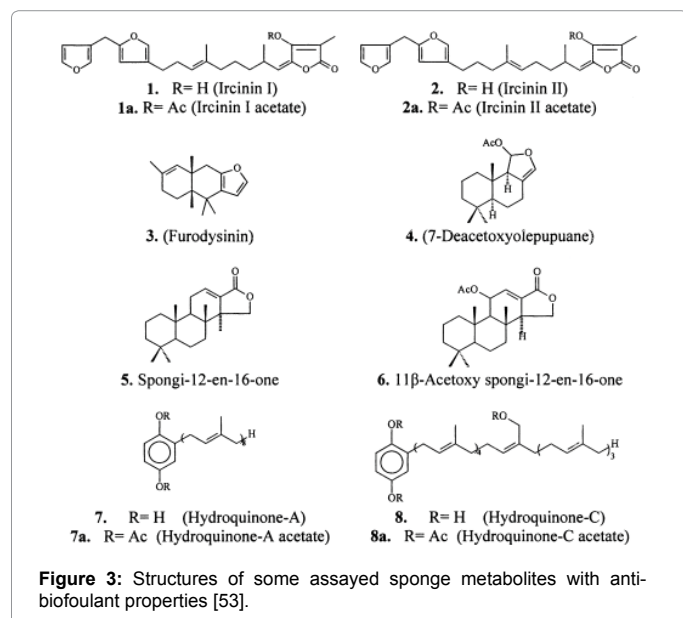
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**Figure 2: Examples of marine invertebrate groups commonly reported in the literature for studying production of anti-fouling repellent substances. a: Coral; b: Gorgonian; c: Sponge; d: Soft coral. (Underwater images from the Red Sea) [72].**



### Chemical Defensive Mechanism against Bio-Fouling

Secondary metabolites are widespread among benthic invertebrates and understanding their functional roles in the producing organism has been under intense study in recent times. The hypothesis that sessile or slow-moving organisms, without obvious escape mechanisms and physical protection, are likely to be chemically defended has recently been explored with greater frequency in the marine environment.

For any long-lived sessile benthic organisms like soft corals and

sea cucumbers, bio-fouling and epibiosis must either be tolerated or overcome due to the setbacks associated with a colonized surface, these include; inhibition of photosynthesis, blockage of filter feeding apparatus, and increased risk of mechanical dislodgement or predation [21]. In response to this, a variety of chemical compounds are secreted by benthic invertebrates like soft corals, sea cucumbers, sponges to prevent surface fouling [22]. Some of these compounds prevent settlement much more effectively than organotin compounds and at the same time are far less toxic [23]. Chemical-based settlement inhibition against bacteria and other bio-foulers has been reported in sponges [24-30], Ascidians [24,31,32] and Cnidarians [30,33-36] and Bryozoans [23,37].

Because they lack physical defences, soft-bodied sessile invertebrates such as soft corals often use a refined chemical weapon as antifouling agents such as terpenoids, steroids, acetogenins, alkaloids, and polyphenolics [38]. These compounds can act enzymatically by dissolving the adhesives, interfering with the metabolism of the fouling organisms (e.g., nervous pathway interference), inhibiting the attachment, metamorphosis or growth, promoting negative chemotaxis, altering the surface of the organisms and as repellents [39-41]. Octocorals (class Anthozoa, subclass Octocorallia, and order Alcyonacea, family Alcyoniidae) were one of the first benthic invertebrate groups that were systematically screened for secondary metabolites (Tursch, 1976). These compounds, especially cembranoid diterpenes [42], have a function in chemical defence, in competition for space (allelopathy) and against fouling [43-46]. These diterpenes e.g., from *Sinularia flexibilis*, although lipophilic, are highly soluble in seawater and as anti-fouling agents are selectively absorbed onto bio-membranes of fouling organisms. Triterpene glycosides from two sponges, *Erylus formosus* and *Ectyoplasia ferox*, were tested for a suite of activities including predator deterrence, bacterial attachment, fouling, and overgrowth by competitors. The results showed a strong inhibition of fouling by invertebrates and algae over a 27-day period [47]. Soft corals may chemically affect the larvae of other corals, interactions at the interface of antifouling and allelopathy. Scleractinian coral recruitment was depressed in a current-dependent directional manner around the soft corals *Sinularia flexibilis* and *Sarcophyton glaucum*, and settlement did not occur on plates containing an extract of *S. flexibilis* [48]. The diterpenes flexibilide, dihydroflexibilide, and sinulariide from *S. flexibilis* were toxic to fertilized eggs of the hard corals *Montipora digitata* and *Acropora tenuis* during the first 24 hr [49]. The Atlantic species *Eudistoma olivaceum* produces a range of over 20 alkaloids termed eudistomins [50]. The antilarval activity of these compounds was traced to a pair of isomeric carboline alkaloids, eudistomins G and H. Both of these compounds from the ascidian were found to inhibit settlement of *Bugula neritina* larvae at 2 µg per square cm due to their toxicity in bioassay trials [51]. Secondary metabolites, such as 1-methyladenine from *Aplysilla glacialis* [25], extracts from *Crambe crambe* [26], as well as aerolithionin and homoaerolithionin from *Aplysina fistularis* [52] showed strong antibacterial and bryozoan larval properties. *Dendronephthya spp.*, a Cnidarian, was also reported to contain horamine (*N*-methyl-4-picolinic acid) that significantly inhibited growth of the co-occurring benthic diatom *Navicula salinicola* [30,33]. The antifouling activity of a series of extracts and secondary metabolites, such as bromopyrrole or diterpene alkaloids, from the epibiont-free Mediterranean sponges, *Ircinia oros spinosula*, *Cacospongia scalaris*, *Dysidea sp.*, and *Hippospongia communis* was investigated by Hellio et al. [53]. A number of the tested metabolites had anti-settlement activity when tested against barnacle, *Balanus amphitrite*, cyprids. The effect of sponge extracts and metabolites on

the settlement of barnacles was tested by Hellio et al. using cyprids of *Balanus amphitrite*. The results of the effectiveness of the sponge extracts or metabolites in inhibiting *B. amphitrite* settlement is presented in Table 1 and Figure 4.

In a study reported by Limnamol et al. [54], thirty six species of sponges collected from the Gulf of Mannar, India, were tested for their inhibitory effect on fouling bacterial strains and cyprids of *Balanus amphitrite*. The results showed that *Fasciospongia cavernosa* and *Petrosia nigricans* had a high significant inhibitory or anti-settlement activity against the fouling bacteria and *Istrochota baculifera* larvae.

The experiment carried out by Yang et al. [55] to test for the anti-fouling property of two- compound extracts (10b-formamidokalihinol-A and kalihinol A) from sponge, *Acanthella cavernosa*, against the bacterial and larval settlement of a major fouling polychaete, *Hydroides elegans*. The results showed that both compounds inhibited the growth of bacteria isolated from the natural environment whereas kalihinol A suppressed larval settlement due to modification of bacterial communities on their surfaces which has influence on larval settlement of fouling organism (Figures 5 and 6).

Blihoghe et al. [56] reported that agelasine derivatives, from sponges and soft corals, inhibited settling of larvae of *Balanus improvisus* in an anti-fouling bioassay as well as the growth of planktonic forms of biofilm forming bacteria, *Staphylococcus epidermidis*.

Several studies conducted have shown that soft corals can yield large quantities of promising antifouling metabolites [57]. Chambers et al. [58] reported that 17.95% of potential antifouling natural compounds are from cnidarians (e.g., soft coral). One of the most promising natural antifouling agent identified so far is an isogosterone isolated from an unspecified *Dendronephthya* [37]. Lai et al. [59] evaluated the anti-fouling property of diterpenoids, designated as sinulariols A-S, from Chinese soft coral *Sinularia rigida* on *Balanus species* and concluded that they inhibited the larval settlement of both *Balanus amphitrite* and *B. neritina*. Pereira et al. [60] and Epifaino et al. [61] showed that the diterpene 11 $\beta$ , 12 $\beta$ -epoxypukalide extract from *Phyllogorgia dilatata*, an octocoral, displayed antifouling property when tested on *Perna perna*

and barnacles. Roper et al. [62] revealed that haliclonyclamine A and halaminol A isolated from the sponge, *Haliclona sp.*, have similar effects on sponge, polychaete, gastropod, and bryozoan larvae by inhibiting their settlement and metamorphosis. Qi et al. [63] demonstrated that subergorgic acid, isolated from a gorgonian, inhibited settlement of larvae of *B. amphitrite* and *B. neritina*, with EC<sub>50</sub> values of 1.2 and 3.2  $\mu$ g/mL respectively and LC<sub>50</sub> values of >200  $\mu$ g/mL. Peters et al. [64] showed that two bromophysostigmines, isolated from the bryozoan, *Flustra foliacea*, inhibited bacterial quorum sensing (QS) and the growth of bacteria, suggesting the presence of potential anti-fouling compounds.

## Discussion and Conclusion

Marine invertebrates are one of the major groups of biological organisms (Porifera, Cnidaria, Mollusca, Arthropoda, Echinodermata, etc.) that are significant for their source of a number of natural products and secondary metabolites with anti-biofoulant properties.

It is reported that the secondary metabolites of some species of basibionts can vary quantitatively and qualitatively, depending on the biogeographical location [65], while other species have similar compositions of these metabolites in different habitats [66]. Fusetani proposed that these organisms secrete chemicals that prevent larvae of other marine organisms from settling and growing on them [67]. From the data presented here, it can be concluded that extracts of the various basibionts control a number of epibionts and bio-foulers from settling on their surfaces. Walls et al. [68] and Shellenberger and Ross [69], reported a negative correlation between the presence of secondary metabolites, the antibacterial activity of the extracts and a reduction of fouling, which might indicate an antifouling function for secondary metabolites. Investigations on the Caribbean sponges, *Erylus formosa* and *Ectyoplasia ferox*, showed that triterpene glycosides has multiple ecological functions to deter predation, microbial attachment, and fouling of invertebrates and algae [47,70]. It was found that the metabolites are apparently restricted to the sponge surface and the biological effect is through direct contact with the sponge rather than by water borne interactions. These results support the hypothesis that

Group I (Active and toxic metabolites/extracts)	LC50 (ppm)	EC (ppm)	
Ircinin I&II	4.7	5.0	
Ircinin I&II acetates	4.9	5.0	
Furodysin	18.1	5.0	
Ircinin oros CH <sub>2</sub> Cl <sub>2</sub> extract	21.7	50.0	
7-Deacetoxyolepupane	106.2	100.0	
Dysidea sp. CH <sub>2</sub> Cl <sub>2</sub> extract	52.5	65.9	
Group II (Non-active and nontoxic metabolites/extracts)	%Survival	%Settlement	Concentration ( $\mu$ g/mL)
Spongi-12-en-16-one	100	60.3	100
Hydroquinone A	100	59.4	100
Hydroquinone C	100	57.3	100
Fasciculatin	100	58.0	100
Dysidea sp. aqueous extract	100	59.1	100
11 $\beta$ -Acetoxyspongi-12-en-16-one	100	53.5	100
Group III (Active and nontoxic metabolites/extracts)	%Survival	%Settlement	Concentration ( $\mu$ g/mL)
Euryfuran	100	24.7	100
Hydroquinone A-acetate	100	19.9	100
Dihydrofurospingin II	100	11.2	100
Hydroquinone C-acetate	100	0.0	10
Dysidea sp. alcohol extract	100	0.0	25

Metabolites/extracts are classified according to their activity on inhibition of settlement. In group I, results are expressed as effective concentration for 0% settlement (EC) and concentration including 50% lethality (LC<sub>50</sub>). For groups II and III, results are expressed as percentage of survival and of settlement for the reported concentrations.

Table 1: Settlement inhibition activity against *Balanus amphitrite* cyprids [53].



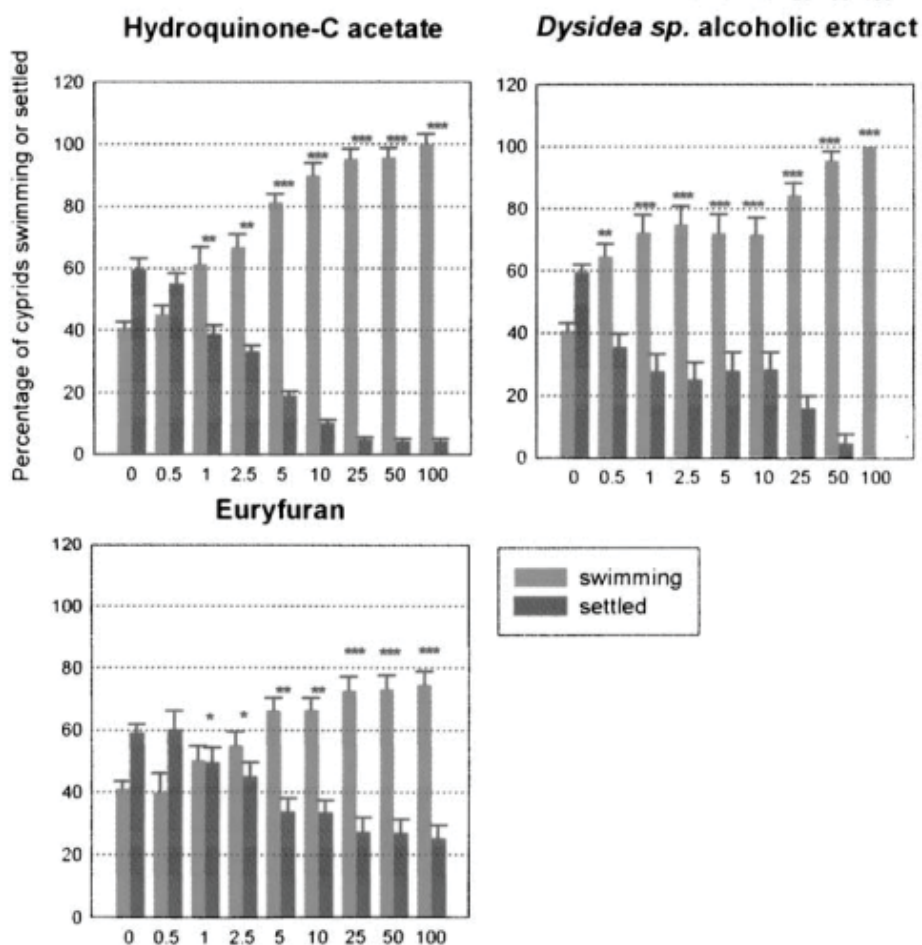


Figure 4: Effect of the most active nontoxic metabolites/extracts (0 to 100 g/mL) on *B. amphitrite* cyprid settlement. Results are expressed as percentage settled ( $\pm$  SEM) and percentage swimming ( $\pm$  SEM). Results significantly different from the control, \*P<0.05; \*\*P<0.001; \*\*\*P<0.001 [53].

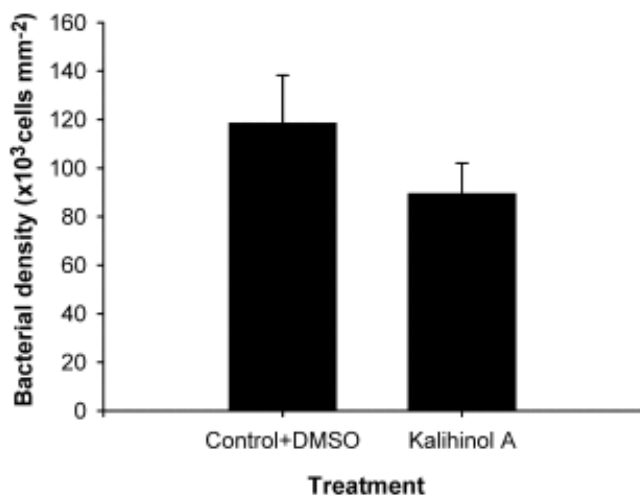


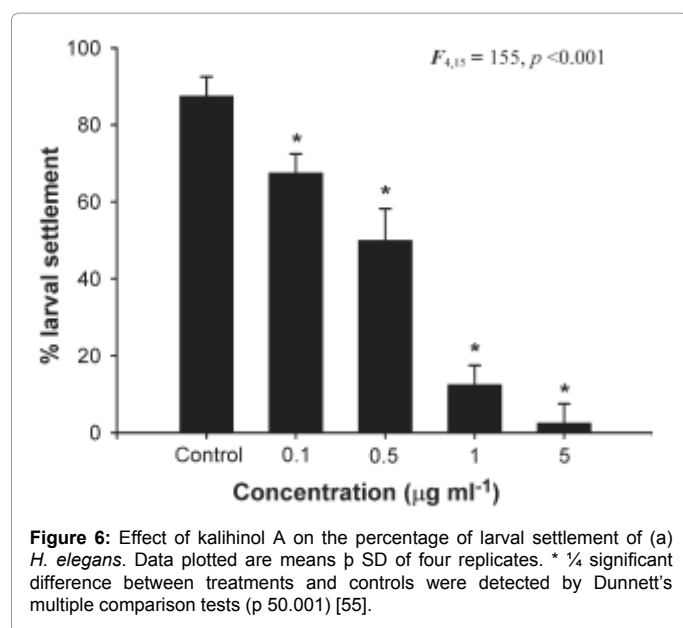
Figure 5: Bacterial density on the surface of Phytagel1 embedded with kalihinol A and the control containing DMSO (n ¼ 3). The Phytagel1 were exposed to flow-through natural seawater for 3 d at 208°C in March 2005 [56]. DMSO: Dimethyl sulfoxide.

invertebrate metabolites are involved in the regulation of microbial and other bio-fouler distributions in the marine environment, and may act as a chemical defence aimed at controlling surface colonization. Therefore, it can now be concluded that surfaces of marine invertebrates, e.g., sponges, soft corals, are usually remarkably free of fouling organisms, supporting the assumption that this is achieved by secretion of anti-fouling compounds [71,72].

A lot of antifouling compounds have been isolated and reported from marine sponges, sea anemones, soft coral, etc but their molecular structures are too intricate to be artificially synthesized. Better understanding of the natural function of these secondary metabolites will to develop new strategies for the correct management and protection of these potentially important natural resources, the basibionts, for the future and find new biotechnological applications for these products in our day-to-day lives. Exploiting these marine biotas could lead to scarce supply of anti-fouling compounds widely used by many industries such as aquaculture, pharmaceutical and shipping.

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