Efficacy of Bioactive Compounds of Sponges to Prevent Biofilm Formation on Medical Implants

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Abstract Bacterial biofilms are surface-attached communities of microorganisms that are protected by an extracellular matrix of biomolecules. In the biofilm state, bacteria are significantly more resistant to external assault, including attack by antibiotics. In their native environment, bacterial biofilms underpin costly biofouling that wreaks havoc on shipping, utilities, and offshore industry. Within a host environment, they are insensitive to antiseptics and basic host immune responses. It is estimated that up to 80% of all microbial infections are biofilm-based. Biofilm infections of indwelling medical devices are of particular concern, since once the device is colonized, infection is almost impossible to eliminate. Importantly, we discuss several sets of compounds derived from marine sponges that we are developing in our labs to address the persistent biofilm problem. Marine bioactive compound synthesis of natural products and their analogues-including our marine sponge-derived compounds and initial adjuvant activity and toxicological screening of our novel anti-biofilm compounds.

Keywords Biofilms, Antifouling, Marine sponges, 2-methoxydecanoic acid, 3-hydroxytetradecanoic acid compound

1 Introduction

Biofouling is a costly and destructive natural phenomenon that affects almost every economic sector from shipping to medicine, causing billions of dollars in damage and disruptions annually. While biofouling is typically linked to aquatic invertebrates, it is the formation of a biofilm that acts as the glue that binds these animals to a surface (Callow and Callow, 2000; 2006). Biofilms can be generated by numerous species of microorganisms but are primarily the creation of bacterial microcolonies that have attached to a surface and shielded themselves in an extracellular matrix of polysaccharide, protein, and nucleic acids (Costerton et al., 1999). Utilizing this line of defense, bacteria have been able to successfully permeate every environmental niche, including the human body. According to the NIH, biofilm-based microbial infections make up to 80% of all infections in human patients, leading the CDC to declare biofilms to be one of the most important medical hurdles of the century (Musk and Jr Hergenrother, 2006).

In an effort to find viable sources of anti-biofilm agents, many researchers have started to extract and analyze natural products from a myriad of plants and marine organisms (Koopmans et al., 2009). Many of these compounds are secondary metabolites that are generated by the host organism in response to external pressures, such as competition for space and potential predators (Wulff, 2006). In a marine environment, antimicrobial and antifouling metabolites are vital for many sessile organisms to ensure that they do not host hazardous biofilms on their exposed surfaces, especially given that an overwhelming majority of the planet’s microbial biomass prefers to be in a biofilm state (Hall-Stoodley et al., 2004). Of all the species studied, marine sponges (Phylum Porifera) have been some of the most valuable. Sponges have been the source of more than 30% of marine natural products, generating a diverse array of molecules that have been found to have not only antimicrobial capabilities but also potential as cancer therapeutics.

Despite their potential, there are very few of these metabolites and their derivatives that can serve as biofilm modulators in a non-microbial manner (Stowe...
et al., 2011). The purpose of this review is to present those compounds derived from sponges that have shown anti-biofilm potential without bactericidal effects, with particular focus on the screening and development of our own chemical libraries as an example. These non-lethal molecules are significant discoveries, because they have the potential to serve as therapeutic supplements that can enhance the efficiency of conventional antibiotics against biofilm-based infections without eliciting resistant phenotypes (Stowe et al., 2011). While it is important to continue to expand our libraries of antibiotic molecules, it is essential for the continued efficacy of all antimicrobial drugs that new and effective adjuvants be discovered in order to mitigate the increasing microbial resistance derived from biofilms (Stoodley et al., 2002).

1.1 Biofilm development and defense

Ever since van Leeuwenhoek noted his belief that the number of organisms found in 17th century dental plaque exceeded “the number of men in a kingdom”, biofilms have been a part of our understanding of how bacteria interact with their environment, but it was not until the late 20th century that we began to realize their true importance. Over the last few decades, intense study of a deluge of bacterial species from diverse environmental niches has lead to a generalized conception of how biofilms are formed (Stoodley et al., 2002). For most species, biofilm formation occurs in five distinct stages (Figure 1) that are defined by a combination of phenotype and genetic changes (Stoodley et al., 2002). It is also during the first stage of attachment that most antifouling and antimicrobial compounds are the most effective, because the bacteria have not coated themselves in the exopolymeric substance (EPS) and are in a vulnerable state (Stowe et al., 2011).

According to this theory, each biofilm contains a small population of highly resistant bacteria that are able to survive antibiotic kill-off to rebuild the biofilm population (Spoering and Lewis, 2001). Although the concept of persister cells has been around over seventy years, it has only been through the recent increase in medical biofilm research that the correlation between biofilm-based disease relapse models and persister cells could have been made (Percival et al., 2011). Since the persister cells are protected, they are able to reproduce once the microbicidal insult abates and yield stronger progeny that have a significantly higher chance of being antibiotic resistant after each treatment, leading to a chronic disease that is driven by antibiotic resistant bacteria found in normal acute infections (Stowe et al., 2011).

1.2 Anti-Biofilm agents derived from marine sponges

Marine sponges can be likened to little factories for bioactive secondary metabolites. These benthic organisms are some of the simplest multicellular animals with little differentiation and long lives, relying on the water around them to supply all their essential needs. Therefore, the generation of chemical defenses is a key element of their survival (Braekman et al., 1992), whether they need to ward off predators, fight off competition for space and resources or control surface fouling (Pawlik, 1993). Sponges utilize a plethora of chemical classes to protect themselves and even to communicate with symbiotic organisms that can provide nutrients and additional protection (Manzo et al., 2011). Many of these chemicals have been found to have antifouling and anti-biofilm properties, but very few have been shown to modulate biofilm formation without killing the bacteria or disrupting their growth. To date, only two classes of marine sponge metabolites house non-bactericidal biofilm modulators, the terpenoids and the pyrrole-imidazoles (Melander et al., 2009). Although not unique to sponges, one of the most potent and diverse groups of molecules is the terpenes and their derivatives.

1.3 Biofilm of medical implants

Because of the importance of bioactive compounds in sponges to inhibit bacterial biofilm formation, in the present study an attempt has been made to find out catheter the extracts of the sponges Aurora globostellata and Spirastrella inconstans var. moeandrina Dendy...
can inhibit microbes biofilm formation on medical implants like urinary catheter.

Catheter associated urinary tract infection (CAUTI) was the second most common nosocomial infection after ventilator associated pneumonia (VAP), affecting 16.3% of the patients with a urinary catheter (Sallam et al., 2005). Despite relatively low mortality rates, CAUTI represent a large problem in hospitals due to additional hospital stay and treatment cost. Nosocomial associated urinary tract infection comprise perhaps the largest institutional reservoir of nosocomial antibiotic-resistant pathogens (Potic and Ignjatovic, 2008). Organisms capable of infecting the urinary tract during catheterization use approaches to establish infection that are similar to those used by organisms that cause uncomplicated UTIs (Jacobsen et al., 2008).

Clinical observations have established that the microbial populations within CAUTI frequently develop as biofilms, directly attaching to the surface of catheters (Trautner and Darouiche, 2004). One main strategy for control and prevention of bacterial adhesion, on medical implant is changing the surface properties of implants to prevent the initial attachment of microorganisms and reduce the number of persistent pathogens (Moons et al., 2009).

Marine bioactive compounds are frequently strong and often are highly specific in their defense activities due to a diversified exposure (Glaser and Mayer, 2009). Though marine organisms are very sessile, they hold a brilliant stock of such anti-microbial metabolites factory to avoid formation of host hazardous biofilm on their own exposed surfaces; provided the overwhelming majority of planets’ microbial biomass prefers to be in a biofilm state (Nicolas et al., 1999). Hence, these organisms can be chosen to isolate the compound that can be used for inhibition of biofilms and for human use. Hence in the present study the extracts of two marine sponges were tested against microbial biofilm formation inhibition in urinary catheters.

2 Results and discussion

Out of the 47 catheters screened, 46 were found with a biofilm formation. The biofilm formation was heavy on the catheters, which were used for a long time. Catheters that were used for over 3 months had a dense biofilm matrix. An examination of biofilm that was detached from the catheter was found to harbour consortia of microbial communities. Seven species of microbial strains were isolated from the biofilm matrix. An assessment of the different microbes that constituted the biofilm revealed that the Escherichia coli was a dominant unit in biofilm (30%) followed by, Proteus mirabilis (28%), Staphylococcus epidermidis (17%) and S. aureus (15%) incidence. The incidence of Candida albicans (11%), Pseudomonas aeruginosa (4%) and Neisseria gonnorhaeae (1%) was found less (Table 1).

Table 1 Microorganisms screened and identified from infected urinary catheters

| Microorganisms          | Occurrence of different microbes in catheter biofilm (%) |
|-------------------------|----------------------------------------------------------|
| Proteus mirabilis       | 30                                                       |
| Staphylococcus aureus   | 28                                                       |
| Staphylococcus epidermidis | 17                                                      |
| Pseudomonas aeruginosa  | 15                                                       |
| Escherichia coli        | 11                                                       |
| Neisseria gonnorhaeae   | 1                                                        |
| Candida albicans        | 4                                                        |

In the present study six bacterial strains viz., Proteus mirabilis, Escherichia coli, Staphylococcus epidermidis, S. aureus, Pseudomonas aeruginosa, Neisseria gonnorhaeae and one fungal strain Candida albicans were isolated from the tips of urinary catheter associated biofilm. The sensitivity of these microbial isolates was tested using standard antibiotics and the extracts of two marine sponges (Ag and Si). Three different doses of the extracts were used to find out the antibacterial activity on biofilm forming microbes. Of the three concentrations tested (20 µL, 40 µL and 60 µL) 60 µL dose of both Ag and Si extracts showed a good inhibitory activity of the extracts of both Si and Ag was high on Proteus mirabilis, followed by S. epidermidis and S. aureus (Table 2).

The maximum inhibition 16.33 mm for Ag and Si was observed. This inhibitory effect was high when compared with standard antibiotics, Tetracycline. Between the two sponges Si and Ag, the inhibitory action varied over bacterial isolates to the extracts of Si was very effective inhibitory (16.33 mm) the growth of C. albicans when compared with Ag (12.33) at 60 µL dose (Table 3).
Table 2 Antibacterial activity of the extract of the sponge *Spirastrella inconstans var.moeandrina Dendy* tested against biofilm isolated pathogens

| Microorganisms                  | Zone of inhibition (diameter) | 20 µL     | 40 µL     | 60 µL     | Tetracycline |
|--------------------------------|------------------------------|-----------|-----------|-----------|--------------|
| *Proteus mirabilis*            |                              | 11.33±0.66| 13.33±0.66| 16.33±0.33| 10.33±0.33   |
| *Escherichia coli*             |                              | 10.33±0.33| 11.00±0.57| 12.33±0.33| 10.00±0.57   |
| *Staphylococcus epidermidis*   |                              | 10.66±0.33| 11.66±0.33| 12.00±0.57| 10.33±0.33   |
| *Neisseria gonnorrhoea*        |                              | 9.33±0.66 | 11.33±0.33| 14.66±0.33| 10.33±0.33   |
| *Staphylococcus aureus*        |                              | 11.00±1.00| 12.33±0.33| 13.00±0.57| 10.33±0.33   |
| *Pseudomonas aeruginosa*       |                              | 9.33±0.66 | 10.33±0.33| 12.33±0.33| 9.00±0.57    |
| *Candida albicans*             |                              | 12.66±0.66| 10.33±0.33| 16.33±0.33| 11.33±0.66   |

Table 3 Antibacterial activity of the extract of the sponge *Aurora globostellata* tested against biofilm isolated pathogens

| Microorganisms                  | Zone of inhibition (diameter) | 20 µL     | 40 µL     | 60 µL     | Tetracycline |
|--------------------------------|------------------------------|-----------|-----------|-----------|--------------|
| *Proteus mirabilis*            |                              | 12.66±0.66| 15.00±0.57| 16.33±0.33| 10.00±0.57   |
| *Escherichia coli*             |                              | 10.66±0.66| 13.33±0.33| 14.66±0.66| 10.66±0.66   |
| *Staphylococcus epidermidis*   |                              | 13.00±0.57| 14.66±0.33| 15.71±0.78| 12.33±0.33   |
| *Neisseria gonnorrhoea*        |                              | 9.33±0.66 | 10.33±0.33| 12.33±0.33| 11.00±0.57   |
| *Staphylococcus aureus*        |                              | 11.00±0.57| 11.66±0.33| 13.33±0.66| 10.66±0.33   |
| *Pseudomonas aeruginosa*       |                              | 10.33±0.33| 13.00±0.33| 15.33±0.33| 10.00±1.00   |
| *Candida albicans*             |                              | 10.33±0.33| 10.66±0.33| 11.33±0.33| 9.66±0.66    |

There are many reports on the effect of the extracts of sponges to prevent microbial biofilm formation on non-medical implants. This has been extensively reviewed (Stowe et al., 2011). According to the previous reports it is understand that the bioactive compounds in the sponges particularly terpene and pyrrole-imidazole, alkaloids are the major antifouling agents. In the present investigation the 16th and 18th fraction of Ag and Si extracts showed the presence of many compounds particularly, 3-hydroxytetradecanoic acid, 2-methoxydecanoic acid. These compounds are also very effective in inhibit the bacterial biofilm.

It is estimated that upto 80% of all microbial infections are biofilm based (Stowe et al., 2011). Biofilm infections of indwellinary medical devices are particular concern since once the device is colonized, infection is almost impotent to eliminate. The present study exposes the potential of the bioactive compounds in sponges to prevent microbial colonization in medical implants. So further study is needed to find out the mechanisms to surface coat the metabolites separated from sponges over medical devices before implanting. This will save a lot of human life.

3 Conclusion

Different doses of the fraction of the sponges were tested to find out thus efficacy to inhibit the growth of bacteria inhabiting urinary catheter. Normally the catheter inoculated bacteria are less responsive to
drugs but such drug resistant isolates were found very sensitive to sponge extracts. From the present study it is concluded that the 16th and 18th fractions of the sponges Aurora globostellata and Spirastrella inconstans var. moenandrina Dendy respectively has rich bioactive compounds and that compound must be identified exactly. This will be a panacea for many pandemic problems.

4 Material and methods
4.1 Isolation of bacteria from catheters
Urinary catheters from patients being cared for in hospitals in Chidambaram, India were collected. Sections of 1–2 cm were cut from the tip of catheter that was removed from patients in whom the catheter was implanted for over 2 months. Forty catheters were used. The tips were suspended in quarter-strength Ringer’s solution in (10 mL) in sterile universal containers, as explained by Sonification for 5 min at 35 kHz in a transonic water bath and by vortex mixing for 2 minutes was used to remove and disrupt the colonizing biofilms. The resulting cell suspensions were cultured onto Cystine lactose electrolyte deficient media (CLED) Chromogenic UTI and tryptone soya agars (Hi-media). After 24 h incubation the resulting colonies were identified using standard procedures. From the catheters seven types of were isolated viz., Proteus mirabilis, Escherichia coli, Staph ylococcus epidermis, S. aureus, Pseudomonas aeruginosa, Neisseria gonnorhaea and Candida albicans.

4.2 Anti-bacterial assay
Disc diffusion method was adopted to screen the anti-bacterial potential of the extract of sponge against biofilm isolates on Luria Bertani (LB) agar plates. This is a valuable and inexpensive test to demonstrate the susceptibility of a particular compound against pathogens by measuring the relative zone of inhibition. To achieve this, sterile antibiotic disks (HIMEDIA Laboratories, India) of 6 mm diameter were loaded with 20 µL, 40 µL, 60 µL of the extract of the sponge A. globostellata keeping a control using Tetracycline. The disks were then air-dried aseptically. Exponential bacterial cultures were swabbed on to the LB agar plates and the disks were left on the agar plates. The plates were incubated at 37°C for 24 h, meanwhile the experiments were performed in triplicates.

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