Antimicrobial Activity of Sponge Associated Macroorganisms Against Fish Pathogen

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Abstract: Antimicrobial potential of sponge associated microorganisms have been dealt for a couple of centuries, but the bioactivity of sponge associated macroorganism have not been attempted so far. The present study was carried out to find out the sponge associated macroorganism and their bioactive potential against chosen fish pathogens. Marine sponges were monthly collected by scuba diving at sampling station viz. Thondi. Five sponge samples viz., Clathria vulpine, Stylissa carteri, Hyattella intestinalis, Clathria indica and Haliclona grant were collected from Palk Strait region. Associated macroorganism viz., Hypnea valentiae sponge and Nereis sp. The bioactive potential of sponge extract and associated macrofaunal and floral extract was showed that, the crude extract from Clathria vulpine showed maximum average zone of inhibition (10.25mm) against Bacillus subtilis and Vibrio harveyi. Among the assoiated macroorganisms, the seaweed Hypnea valentia and Nereis sp showed maximum average zone of inhibition (9.25 and 7.5mm). The overall antimicrobial activity assessed from the above results indicated the presence of active constituents in the extractions of sponges and assoiated macroorganisms which can be exploited for the production of lead molecules which are of use in pharmaceutical industry.

Key words: Antibacterial activity, Sponges, Associated macroorganisms, Fish pathogens

INTRODUCTION

Sponges (Phylum Porifera) are evolutionarily ancient metazoans that have existed for 700–800 million years. They not only populate the tropical oceans in great abundance, but also occur in temperate waters and even in freshwater [1-2]. Marine sponges are widely distributed from intertidal zones to thousands of meters deep in the ocean [3]. They are simple multicellular invertebrates attached to solid substrates in benthic habitats. Sponges are filter feeders, having numerous tiny pores on their surface, which allow water to enter and circulate through a series of canals where microorganisms and organic particles are filtered out and eaten [4]. There are mainly three classes of sponges, namely the Calcarea (five orders and 24 families), Demospongiae (15 orders and 92 families) and Hexactinellida (six orders and 20 families). So far about 15,000 species of sponges have been described, but their true diversity may be higher [5]. Most of them occur in the marine environment and only about 1% inhabit freshwater [6]. Most of the species are placed under the class Demospongiae. Since sponges are simple and sessile organisms; during evolution they have developed potent chemical defensive mechanism to protect themselves from competitors and predators as well as infectious microorganisms. Studies show that secondary metabolites in sponges play a crucial role in their survival in the marine ecosystem [7]. Sponges have bioactive potential by the presence of active ingredients preserve in sponges themselves or their microbial symbionts of chemicals that may be used to control viruses, bacteria, tumors and fungi [8]. Previous studies on mangrove plant parts and its major chemical classes displayed various level of biological activities such as antibacterial, antifungal, antiplasmodial, cytotoxic, antifouling, hepatoprotective, ichtyotoxic, cytotoxic and free radical scavenging activities [9-16]. These natural products have interesting biomedical potential, pharmaceutical relevance and diverse biotechnological applications [17]. The biomedical and pharmaceutical importances of these compounds are attributed to their antiviral, antitumor, antimalarial, antimicrobial and general
cytotoxic properties [18]. Moreover, the bioactive potential of associated microorganism has been elaborately studied, but so far, no such studies have been undertaken to study the bioactive potential of sponge associated macroorganisms. The present study, described the efficacy difference among the sponge associated macroorganisms against bacterial fish pathogens.

**MATERIALS AND METHODS**

**Collection of Sponges:** Marine sponges were monthly collected by scuba diving at sampling station viz Thondi. (latitude of 99°44”N and 79 10’ 45”E). Five sponge samples were collected from Palk Strait viz., Clathria vulpine, Stylissa carteri, Hyattella intestinalis, Clathria indica and Haliclona grant normally the collection was carried out around 8 AM in the morning. Sample collections were carried out for a period of three month from the Palk Strait. All the samples were sealed in a pre-sterile plastic bag individually and stored in a defreezer.

**Identification of Sponge Associated Macroorganism:** After collection, the sponge samples were thoroughly washed with tap water without disturbing the associated macro-organisms. Then the associated macro-organisms were separated from the sponge tissue and examined for species level identification by following standard identification manual. Associated macroorganism viz., Hypnea valentiae and Nereis sp.

**Extraction of Bioactive Principles:** 400mg of associated macro-organisms viz., seaweed, neries and sponge samples were ground well with 3 ml of sterile distilled water and then centrifuged at 10,000 rpm for 10 minutes. The supernatant were collected and lyophilised and further stored it in refrigerator for further use.

**Antimicrobial Sensitivity Assay:** Filter paper disc method was used to carry out the antimicrobial sensitivity assay. Lyophilised extracts were impregnated on to sterile filter paper disc and placed on to the Mueller-Hinton agar (Himedia,Mumbai) which were previously swabbed with bacterial fish pathogens viz., Bacillus subtilis, Aeromonas hydrophyla, Vibrio parahaemolyticus, Serratia sps and V.harveyi. Control disc was maintained without the extracts. All the plates were incubated overnight at 37°C under static conditions. After that, the zone of inhibition appearing around the discs were measured and recorded as zone of inhibition in millimeter in diameter. Triplicate samples were maintained for each bacterial strain.

**RESULT AND DISCUSSION**

The present study made an attempt to find out the bioactive potential of sponge extract and associated macrofauna and flora. It showed that, the crude extract from Clathria vulpine show maximum average zone of inhibition (10.25mm) against chosen fish pathogen (Table 1). Among the fish pathogens Bacillus subtilis and Vibrio harveyi was severely affected with the sponge and associated macroorganism. It is also interesting to notice that, the sponge and associated seaweed showed maximum sensitivity than the associated fauna Neries. Sponge-microbial associations are found to be very specific in the production of particular bioactive compounds. However, the mutual mechanism between host and the microbial associate, in compound production is not well understood. The easiest and best way for commercial production of these compounds are either by culturing the host and/or the associated microbe under controlled conditions. But, the ability of the symbiont to produce the compound consistently for several generation in culture media has to be tested and standardized [19]. Sponges have proved to be a rich source of novel chemicals, many with potential use in treatment of human disease and in industrial applications. The increasing incidence of cancer in western society, development of antibiotic resistance amongst human pathogenic bacteria and the advent of viral diseases such as HIV/AIDS are just three reasons for the growing interest in marine organisms as sources of new drugs with antibacterial, antiviral, anticancer and other pharmacological activities. Concurrent with discoveries of novel sponge chemicals has become evidence that many of the novel metabolites found in sponges are microbial in origin and result from the activities of microbes,
Table 1: Antibacterial activity of sponge and associated macrofauna and flora against 5 fish pathogens

<table>
<thead>
<tr>
<th>Sponges associated organism</th>
<th>A.hydrophila</th>
<th>V.parahemolyticus</th>
<th>B.subtilis</th>
<th>Serratia sp</th>
<th>V.harveyi</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neries sp.</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>-</td>
<td>7.5</td>
</tr>
<tr>
<td>Clathria vulpine (Sponge)</td>
<td>9</td>
<td>8</td>
<td>12</td>
<td>-</td>
<td>12</td>
<td>10.25</td>
</tr>
<tr>
<td>Hypnea valentia (Seaweed)</td>
<td>7</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>-</td>
<td>9.25</td>
</tr>
<tr>
<td>Average</td>
<td>7.7</td>
<td>9.0</td>
<td>12</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

particularly bacteria, living symbiotically within the tissues of the sponge. The present study clearly showed that the sponge species of *Clathria vulpine* showed better antibacterial activity against fish pathogens particularly *Bacillus subtilis* and *Vibrio harveyi*. Microorganisms play a central role in sponge biology: they serve as food particles and are found to live associate with many sponges inter- and intracellularly [20, 21]. Several observations support the idea that bacteria synthesize sponge-specific compounds either completely or in the form of precursors completed subsequently by sponge metabolism [22 –25]. Antimicrobial and other biological activities of associated bacteria may play a significant ecological role in sponge–bacteria associations. Furthermore, the isolation of sponge-associated bacteria producing bioactive metabolites, which were originally isolated from sponges, strongly supports the hypothesis of the microbial origin of the compounds formerly ascribed to sponges [26, 27]. Sponge associated microorganisms are responsible for the bioactivity than the host sponges. It is true why it is not in the case of sponge associated macroorganisms?. From the present study, it was evident that, sponges are responsible for the bioactivity than the sponge associated organisms. The biologically active metabolites from sponges are either from the sponges or sponge associated organisms is still in debate. It can be cleaned when the technology for the axenic culture of sponges under captive conditions is developed unless impossible to arrive concrete conclusion.

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REFERENCES


