

## Antibacterial Activity of Marine Sponge Extracts against Fish Pathogenic Bacteria

G. Annie Selva Sonla<sup>1\*</sup>, A.P. Lipton<sup>2</sup> and R. Paul Raj<sup>1</sup>

<sup>1</sup> Central Marine Fisheries Research Institute, Cochin, 682018, Kerala, India

<sup>2</sup> Center for Marine Science and Technology, Rajakamangalam, 629502 Tamil Nadu, India

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### Abstract

Secondary metabolites of the marine sponges *Acanthella elongata*, *Axinella donnani*, *Callyspongia diffusa*, *Callyspongia subarmigera*, and *Echinodictyum gorgonoides* were collected from fishing nets and their *in vitro* antibacterial properties against eight virulent marine fish pathogens were studied at incubation temperatures of 20°C and 30°C. Crude methanol extracts of the tested sponges showed species-specific antibacterial activity. The most active species was *A. elongata* which inhibited 100% and 87.5% of the tested bacterial isolates at 20°C and 30°C, respectively. *Callyspongia subarmigera* was the least active as it inhibited only 62.5% and 50% of the tested bacteria at those temperatures. Results suggest that fractionation and purification of the crude methanol extract of *A. elongata* has potential in the development of novel antibiotic substances for managing common bacterial diseases in aquaculture.

### Introduction

The evolving resistance of microorganisms to existing antibiotics has made necessary a search for new antibiotics for human as well as aquacultural purposes. In the aquatic environment, competition for space and nutrients leads to evolution of antimicrobial defense strategies. This, along with possibly adverse effects on the ecosystem and human health problems, has resulted in restrictions on the use of commercial antibiotics and chemicals in the aquatic environment (Serrano, 2005). Bacteria that are resistant to antibiotics must be tackled with more effective and safer

antibiotics. Such substances include natural products derived from marine organisms (Selvin and Lipton, 2004).

Sessile, soft-bodied marine invertebrates such as sponges lack obvious physical defenses and biosynthesize bioactive non-primary or secondary metabolites to protect themselves and maintain homeostasis (Attaway and Zaborsky, 1993). Sponges have antibacterial, antifungal, antiviral, anti-helminthic, anticoagulant, antitumour, cytotoxic, antidiabetic, antiinflammatory, antimalarial, antiplatelet, antiprotozoal, antileukemic, anti-

\* Corresponding author. E-mail: anniesolomon@gmail.com

tuberculosis, and immunomodulatory activities (Butler, 2004). Considering their scope of antibiotic activity against fish pathogenic bacteria, marine sponge extracts are prime candidates as sources of bioactive metabolites.

Sponges are potential sources of antibacterial secondary metabolites that are part of their defense and survival in the marine environment (Matsunaga et al., 2001). Antibiotics in sponges can be used as offensive or defensive mechanisms, offensive when involved in feeding and defensive to prevent infections (Green, 1977). The presence of antibacterial compounds in sponge extracts was studied using assays to detect the inhibition of bacterial growth or cell death (Newbold et al., 1999). The present study evaluated the ability of five marine sponge extracts to inhibit the growth of eight fish pathogenic bacteria so as to use them as alternatives to conventional antibiotics in aquaculture.

#### Materials and Methods

**Collection of sponges.** Sponges were obtained as entangled specimens from fishing nets set at depths of 10-15 m off Kanyakumari (8°04' N, 77°36' E). The churning seawater in this area during November-January and May-August lead to dislodging and entanglement of marine organisms in nets. Fresh bycatches were collected during these periods in 2005-2006, washed in filtered seawater, and blotted. Five species of sponges, selected by their similarities in morphology and color pattern, were identified as *Acanthella elongata* (Dendy), *Axinella donnani* (Bowerbank), *Callyspongia diffusa* (Ridley), *Callyspongia subarmigera* (Ridley), and *Echinodictyum gorgonoides* (Dendy), all of which belong to the class Demospongiae Sollas.

**Extraction of metabolites.** Small pieces of each sponge species were cut and immersed in methanol at room temperature for two days. The sponges were extracted thrice with distilled methanol and the pooled organic solution made from each species was filtered by suction through a Buchner funnel lined with Whatman No. 1 filter paper. Solvents were removed by rotary evaporator (Buchi-type) under reduced pressure to obtain the crude

methanol extract. The methanol was evaporated and the crude extracts were screened against eight species of fish pathogens, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *V. anguillarum*, *V. fischeri*, *V. fluvialis*, *V. pelagius*, and *V. vulnificus*.

**Antibacterial assay.** The standard disc assay given by Bauer et al. (1966) was used. Inocula of overnight cultures of each bacterial strain ( $10^6$  bacteria/ml) were swabbed onto preset Mueller-Hinton agar plates supplemented with 2% NaCl. A 6-mm disc (HiMedia) was impregnated with 30  $\mu$ l of sponge extract and aseptically placed and incubated at  $20 \pm 2^\circ\text{C}$  or at  $30 \pm 2^\circ\text{C}$  in a BOD incubator (Rotek) for 24 h. Discs with the extraction solvents (30  $\mu$ l) served as controls. The diameters of the clear halos around the discs were measured and defined as zones of inhibition.

#### Results

Secondary metabolites of *A. elongata* most successfully inhibited growth of the eight bacteria at both temperatures with inhibition zones of 10-25 mm except for *V. pelagius* which was totally resistant at  $30^\circ\text{C}$  (Fig. 1). *Axinella donnani* inhibited the growth of all bacterial species (9-15 mm) except *V. pelagius* at  $20^\circ\text{C}$ . The *C. diffusa* extract was inactive against *V. fischeri*, *V. vulnificus*, and *V. pelagius* at  $20^\circ\text{C}$  while *V. fluvialis* and *V. pelagius* were resistant at  $30^\circ\text{C}$ . The *C. subarmigera* extract was least active and inhibited only 62.5% and 50% of the tested bacteria at  $20^\circ\text{C}$  and  $30^\circ\text{C}$ , respectively. The *E. gorgonoides* extract exhibited moderate activity (11-16 mm); *V. fischeri* and *V. pelagius* were resistant at  $30^\circ\text{C}$ .

#### Discussion

In the present study, eight fish pathogenic bacteria were susceptible to almost all the tested sponge extracts. Likewise, crude extracts from *Dendrilla nigra*, *A. donnani*, and *Clathria gorgonoides* collected off the Kanyakumari coast as entangled specimens from fishing nets inhibited the common fish and shrimp pathogens *A. hydrophila*, *P. aeruginosa*, *V. alginolyticus*, *V. fischeri*, and

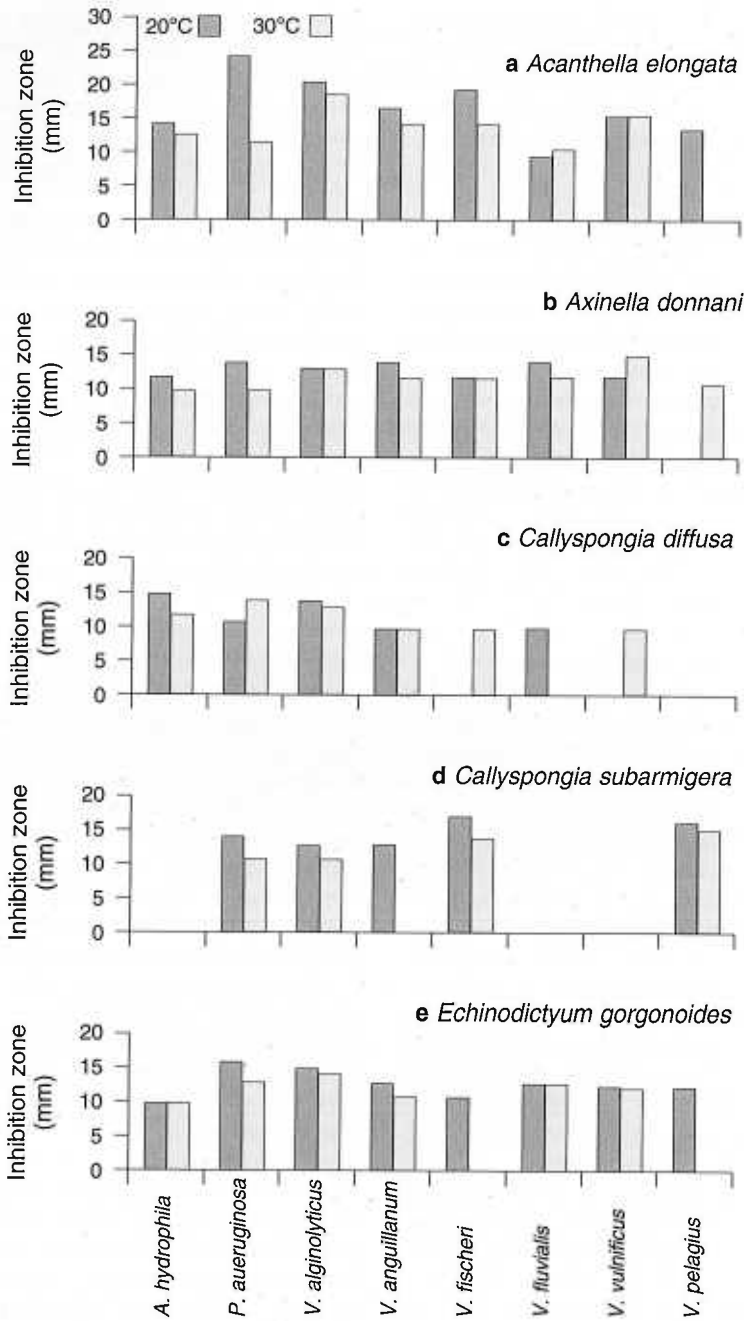


Fig.1. Antibacterial activity of (a) *Acanthella elongata*, (b) *Axinella donnani*, (c) *Callyspongia diffusa*, (d) *Callyspongia subarmigera*, and (e) *Echinodictyum gorgonoides* against eight fish pathogens.

*V. harveyi* (Selvin and Lipton, 2004). *Hippospongia communis* collected from the Atlantic coast exhibited antibacterial activity against the ichthyopathogenic strain of *V. anguillarum* (Pinho et al., 2006).

*Acanthella elongata* was the most important as it inhibited 100.0% and 87.5% of the tested bacterial isolates at 20°C and 30°C, respectively. Sponges classified under Demospongiae produce the largest number and diversity of secondary metabolites with potent antibacterial compounds (Faulkner, 1998). In general, *Acanthella* species exhibit a potent broad spectrum of antibacterial activity against pathogenic bacteria. For example, methanol-toluene (3:1 v/v) and a methylene chloride extract of *A. kleutha* had antibacterial activity against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* but no activity against *Escherichia coli* and *P. aeruginosa* (McCaffrey and Endean, 1985). *Acanthella acuta* exhibited moderate activity against the marine bacteria *Alteromonas luteo-violacea*, *Plesiomonas shigelloides*, *Pseudomonas piscida*, *Serratia marino rubra*, and *V. alginolyticus* (Amade et al., 1987) and *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* (Xue et al., 2004). *Acanthella ramosa* and *A. cavernosa* from the Bay of Bengal were active against the virulent fish pathogens *A. hydrophila*, *Edwardsiella tarda*, *P. aeruginosa*, *P. fluorescens*, and *V. alginolyticus* (Choudhury et al., 2003). Kalihinols, multifunctional diterpenoid antibiotics from *Acanthella* spp., have been isolated (Chang et al., 1987). Considering the high activity profile of the *A. elongata* extract in our study, further fractionation and purification could produce new antibiotic compounds against fish pathogens.

The antibacterial activity of the sponge secondary metabolites was considerably influenced by the incubation temperature. Most of the bacteria were susceptible to the sponge extracts at 20°C. It is possible that the growth of the bacteria was slower and the activity of the sponge metabolites greater at 20°C than at 30°C. It is also possible that, at 30°C, the proliferation rate of the bacteria was faster than the percolation rate of the sponge

metabolites. At the lower temperature, the antibacterial compounds percolated into the agar media more quickly, resulting in a larger inhibition zone. Further studies may provide information about the mechanisms that control zone inhibition in different temperatures.

The results of the present study suggest the potential of sponge metabolites as antibiotic substances to manage common bacterial diseases occurring in aquaculture.

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