SCREENING SPONGES FOR BACTERIOCIDE TO BE USED IN SHRIMP CULTURE

Taufik Ahmad*), Emma Suryati*) and Muliani*)

Abstract

Sponges are suspected of having potential for producing bioactive material that can be used for various purposes. The experiment aimed at identifying sponges which could produce bioactive materials for fish health management purposes. The screening of bioactive extracts from 25 species of sponge was conducted by bioassay test using bacteria such as *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp., *Acinetobacter* spp., and Enterobacteriaceae as bioindicators. Fractionation using different solvents i.e., hexane, acid ethyl acetate, natural ethyl acetate, and water was carried out to obtain isolates of bioactive substant. Based on screening results, 1. bioactive extract from *Halichondria cartilagena* inhibits the growth of *Vibrio* spp., 2. bioactive extract from *Callyspongia pseudoreticulata* prove to be a good inhibitor for *Aeromonas* spp., Enterobacteraceae, and *Pseudomonas* spp., 3. bioactive extract from *Auletta* spp. inhibited the growth of *Acinetobacter* spp., 4. the active fraction extracted from *H. cartilagena* and *Auletta* spp. was polar bioactive 5., from *C. pseudoreticulata*, the active fraction toward *Pseudomonas* spp. was acid ethyl acetate semipolar bioactive and toward Enterobacteriaceae and *Aeromonas* spp. was natural ethyl acetate semipolar bioactive. Bioactive isolates extracted from sponges could be promising for bacteriocides, especially in fish culture.

KEYWORDS: Sponges, bioactive, bacteriocide, shrimp culture

Introduction

Sponges are one of the biological components of coral reefs. Several sponges have been reported to contain bioactive substances which can be used for medical and pharmaceutical purposes. Among them are *Hyatella intestinalis* (Karuso *et al.* 1989), *Algillus flabelliformis* (Gunasekara *et al.* 1989), *Hippospongia comunnis, Spongia officinalis, Ircinia virabilis, Spongia gracillis* (Madaio *et al.* 1989), *Dysidea avara* (Crispino *et al.* 1989), *Erylus lendenfeldi. Dyctionella incisa* (Cimminielo *et al.* 1989). It seems that sponges have potential for producing bacteriocides which can be applied to different purposes, including shrimp and fish culture.

*) Researchers at Research Intitute for Coastal Fisheries, Maros

Expansion of fish and shrimp culture brings increase attention to problems caused by diseases. *Vibrio harveyi* and *Vibrio alginoliticus*, *Aeromonas* spp., *Pseudomonas* spp., and Enterobacteriacea are among the bacteria causing mass mortality of cultured shrimp (Atmomarsono *et al.* 1994; Madeali *et al.* 1994). Rukyani (1994) reported that bacteria caused shrimp cultured losses worth more than US$ 500 million in 1993. To overcome further mass mortality, most of the shrimp farmers applied antibiotics such as chloram-phenicol, oxytetra-cyclin, and prefuran (Brown 1989). Unfortunatelly, the use of antibiotics has been proven to be ineffective and may create problems through stimulating development of immunity to the pathogens.

Realising the harm of using antibiotik, Hadiman (1982) and Suryati (1993) used active ingredients of some fruits and plants to eradicate pathogens from shrimp ponds. However the toxicity of such ingredients is distinctively affected by water salinity and temperature. Acumulation of organic matter in cultures may also cause problems. Since sponges is originated from the sea and share the same ecosystem with shrimp, the bioactive extract may be assumed to have active ingredients which could be used as bacteriocides and would not harm the environment. This research aims at finding the sponges which have the potential to produce bacteriocides.

Material and Methods

The sponges were collected from Spermonde (South Sulawesi) coastal waters. The fresh samples were submerged in organic solvents and kept at 18°C. The identification of genus and species was based on morphological characteristic (Barnes 1990; Brusca 1990) and carried out at Research Institute for Coastal Fisheries laboratory. Masceration in methanol was applied for 72 hours to obtain 600 ml crude extracts from 200 g of blended sponge which then be evaporated to 200 ml.

Fractionation of the samples was carried out using different solvents recomended by Harborne (1973) as shown in Figure 1. The bioindicators used for bioactive screening were *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacteriaceae* which were isolated from diseased shrimp. Bioassay testing of crude extract and of each fraction was carried out using the agar diffusion method as modified by Cowan (1985).

Results and Discussion

Among 25 crude extracts of sponges tested toward Enterobacteriaceae, *Vibrio* spp., *Aeromonas* spp., and *Acinetobacter* spp. Crude extracts of 3 species have been proved to be good inhibitors for all of the bioindicators. Extracts

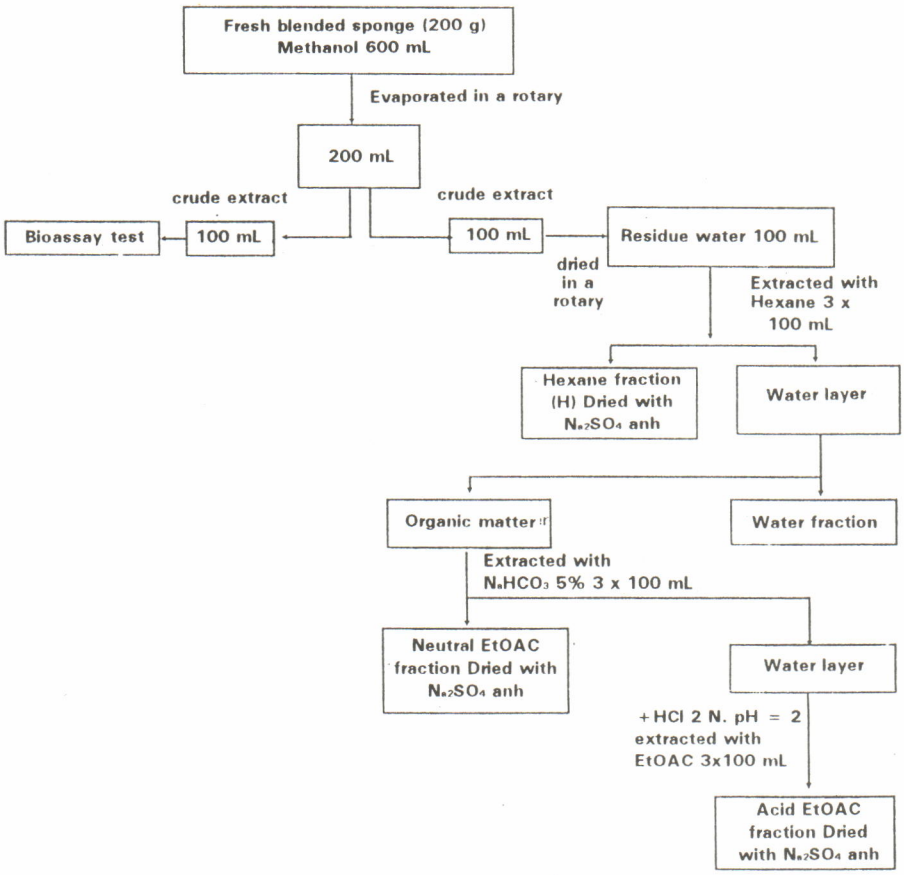

Figure 1. The fractionation flow of sponges bioactive

from 2 species could inhibit the growth of *Acinetobacter* spp., and extracts from Enterobacteriaceae and 1 species could only inhibit the growth of Enterobacteriaceae. Besides, extracts from 10 species inhibited growth of *Vibrio* spp., 9 species inhibit *Aeromonas* spp., and 2 species inhibit Enterobacteriaceae but in a weaker resistance compared to the other 5 species (Table 1). There were 8 species that did not produce extracts which could restrain the growth of bioindicators used.

The differences between biological and chemical characteristics of bio-indicators produce different inhibition capabilities for the crude extracts from sponges. Crude extract of *H. cartilagena* (Figure 2) is highly capable of

Table 1. Inhibition zone (mm) of bioactive extract from sponges toward several bioindicators isolated from cultured shrimp (*Penaeus monodon*)

Sponge species	*Bioindicators*				
	Vibrio spp.	*Aeromonas* spp.	*Pseudomonas* spp.	*Acinetobacter* spp.	Enterobacteriacea
Asteropus sarassinorum	-	-	-	-	-
Auletta spp.	22.5-24.5	14.4-22.2	15.0-23.5	28.1-29.4	24.6-27.9
Callyspongia spp.	+	+	-	-	-
Callyspongia pseudoreticulata	16.6-23.6	22.6-27.4	26.4-31.0	24.4-33.4	20.1-30.1
Cinachyra spp.	-	-	-	-	-
Clathria spp.	-	+	-	-	-
Clathria spp.	+	+	-	-	-
Conyspongia pseudoreticulata	-	+	-	-	-
Cribrochalina spp.	-	-	-	-	12.4-18.3
Desmopsomma spp.	-	-	-	-	-
Dysdea spp.	+	-	-	-	-
Enchynodyctium spp.	-	-	-	-	-
Geliodes spp.	+	+	-	-	-
Halichondria cartilagina	20.3-29.4	16.2-18.8	22.9-24.9	18.6-25.1	20.4-29.1
Helicloma spp.	-	+	-	-	-
Jaspis spp.	-	-	-	-	-
Pericarax spp.	+	-	-	-	-
Phokelia flabelata	+	-	-	-	-
Phyllospongia spp.	-	-	-	-	-
Plakortis nigra	-	-	-	12.2-13.1	12.4-16.4
Strongylacidone spp.	+	-	-	-	+
Thalyasias vulpina	-	-	-	-	-
Theonella cylindrica	+	+	-	-	-
Unidentified sponge	+	+	-	16.2-21.4	14.2-21.4
Xestospongia exipua	+	+	-	-	-

+ : Weak inhibition capability
- : No inhibition

inhibiting the growth of *Vibrio* spp. *C. pseudoreticulata* (Figure 3) produces a bioactive material substances which is strongly inhibits growth of *Aeromonas* spp., *Pseudomonas* spp., and Enterobacteriaceae. Crude extract of *Auletta* spp. (Figure 4) actively restrains the growth of *Acinetobacter* spp.

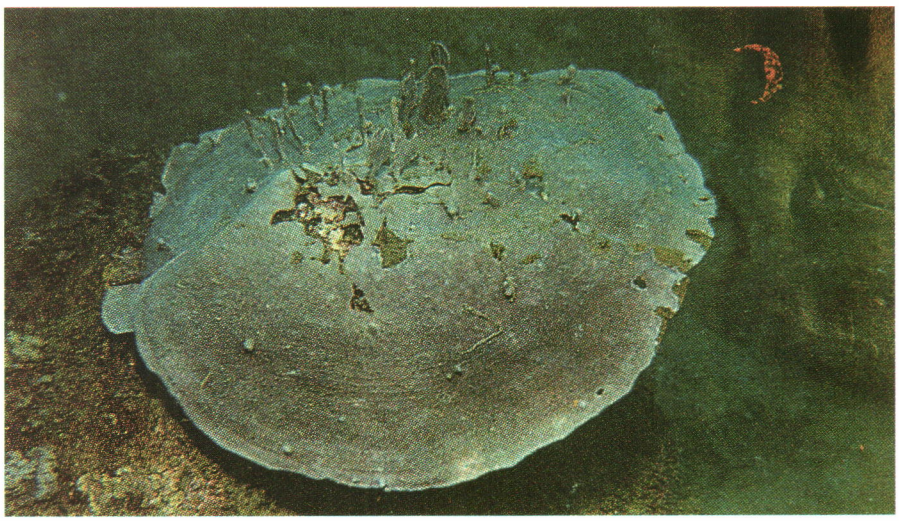

Figure 2. *Halichondrida cartilagena*

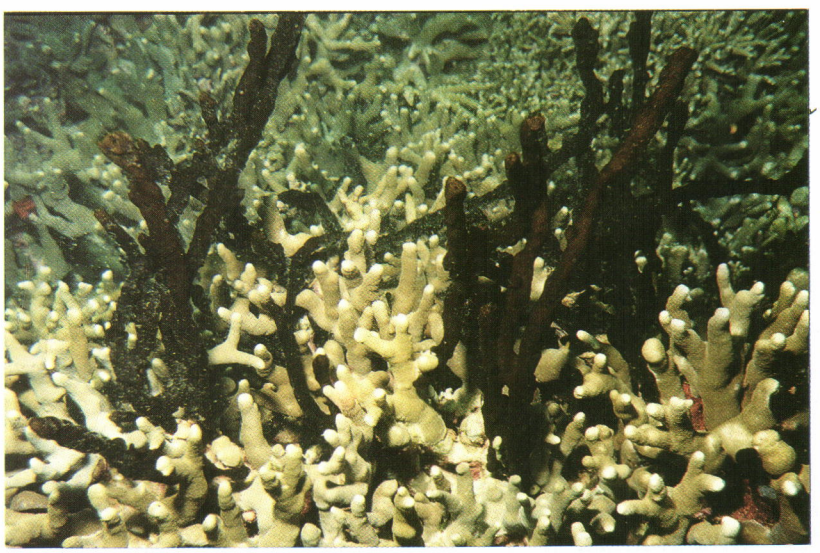

Figure 3. *Callyspongia pseudoreticulata*

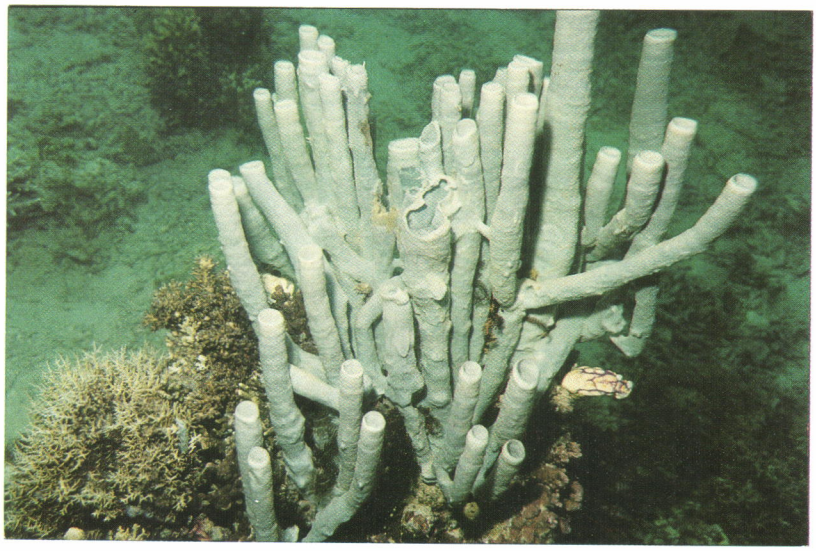

Figure 4. *Auletta* spp.

Fractionation of the 3 species of sponges based on their polarity and solubility produces 4 fractions, i.e., hexane, neutral ethyl acetate, acid ethyl acetate, and water fraction. For *H. cartilagena*, the water fraction is the only one active in inhibiting the growth of *Vibrio* spp. (Table 2). The purity of bioactive substances contained in the water fraction increases its inhibition capability.

Table 2. The capability of bioactive extracts from *H. cartilagena* to inhibit growth of *Vibrio* spp.

Active fraction	Inhibition zone (mm)
Crude extract	20.3-29.4
Hexane fraction	(-)
Acid ethyl acetate fraction	(-)
Neutral ethyl acetate fraction	(-)
Water fraction	25.6-29.6

Note: (-) no inhibition

The water fraction of bioactive extract from *Auletta* spp. inhibits the growth of *Acinetobacter* spp. in an inhibition zone range is from 28.3 to 35.0 mm (Table 3). The bioactive extracts from both *H. certilagena* and *Auletta* spp. are very polar and dissolve completely in water. Among the polar compounds which have the potential to be used as bacteriocides are peptides, glicocides, amines, saponins, and macromolecule carbohydrates (Harborne 1973).

Table 3. Inhibition capability of bioactive extracts from *Auletta* spp. toward the growth of *Acinetobacter* spp.

Active fraction	Inhibition zone (mm)
Crude extract	20.3-29.4
Hexane fraction	(-)
Acid ethyl acetate fraction	(-)
Neutral ethyl acetate fraction	(-)
Water fraction	25.6-29.6

Note: (-) no inhibition

C. pseudoreticulata differs from *H. certilagena* and *Auletta* spp. and produces semipolar bioactive dissolved in ethyl acetate. The acid and neutral ethyl acetate fraction of the inhibits growth of *Aeromonas* spp., *Pseudomonas* spp., and Enterobacteriaceae. The largest inhibition zone was produced by the neutral ethyl acetate fraction toward Enterobacteriaceae (34.0-43.0 mm) followed by acid ethyl acetate fraction toward *Pseudomonas* spp. (Table 4). For *Aeromonas* spp., neutral ethyl acetate fraction is more effective than does acid ethyl acetate fraction.

Table 4. Inhibition zone (mm) of bioactive extracts from *C.pseudoreticulata* toward the growth of bacteria.

Active fraction	Inhibition zone (mm)
Crude extract	28.1-29.4
Hexane fraction	(-)
Acid ethyl acetate fraction	(-)
Neutral ethyl acetate fraction	(-)
Water fraction	28.3-35.0

Note: (-) no inhibition

Chemical compounds dissolved in neutral ethyl acetate are neutral, just like alkaloid compounds such as terpenes, triterpenes, policetida, ketosteroids, and squalene derivates. Acidity compounds dissolved in acid ethyl acetate are acidic, e.g. phenolic acid, carboxylic acid, flavonoids, phenyl propanoids, antocyanins and their derivates (Horborne 1973).

Bacteriocides inhibits growth of bacteria through cell wall irritation, protein coagulation due to pH difference, and hydrolysis and diffusion of cell plasm resulting from osmotic presure imbalance (Salle 1961). Among chemical compounds, peptides, glycocides, terpenoids, steroids, saponins, alkaloids, phenolic acid, alcohol, amines, and organic compound containing metal have the capability to inhibit the growth of bacteria (Salle 1961). Antibiotics and chemical substant such as oxytetracycline, chloramphenicol, malachite green, and prefuran are less effective bacteriocides in fish culture than bioactive sponges (Table 5). At its allowable maximum concentration oxytetracyclin is only capable producing an inhibitation zone of 22.9 mm for *Vibrio* spp. and 19.0 mm for Enterobacteriaceae (Madeali 1995). Chloramphenicol could only inhibit the growth of *Vibrio* spp. and Enterobacteriaceae at 24.6 and 11.9 mm, respectively (Madeali 1994). Malachite green and prefuran at their maximum allowable concentrations are completely ineffective for either *Vibrio* spp. or Enterobacteriaceae.

Table 5. Inhibition capability (mm) of bioactive sponge extracts and antibiotics toward *Vibrio* spp. and Enterobacteriaceae

Chemical compound	Bacteria	
	Vibrio spp.	Enterobacteriaceae
H.certilagena bioactive (20 ppm)	29.6	-
Auletta spp. bioactive (20 ppm)	35.0	-
C.pseudoreticulata spp. bioactive (20 ppm)	-	43.0
Oxytetracycline, (100 ppm)	22.9	19.0
Chloramphenicol, (100 ppm)	24.6	11.9
Malachite green, (100 ppm)	-	-
Prefuran (100 ppm)	-	-

Note: (-) no inhibition

Bioactive sponge extracts is suspected of containing peptide, terpenoid, glycocide, saponin, steroid, amine, phenolic acid and squalene and its derivate produced from secondary metabolism. In nature, sponges utilize bacteria as the source of nutrients (Buss 1976), and formbioactive material during synthetise the digestion process. Barnes (1990) reported that sponges are able to filter the

surrounding bacteria and 77% of its ommision are enzymatically digested. Specific feeding habits of sponges are suspected to effect the bioactive properties which is a used in digestion process. Consequently sponge bioactive is only affective to restrain the growth of bacteria which is part of sponges main diet. In the others words, bioactive sponge extracts provide a species specific bacteriocides.

Conclusion

Two (*H. certilagena* and *Auletta* spp.) out of three sponges examines for bacteriocides produced specific species bioactive substances and the other one (*C. pseudoreticulata*) has the potentiality to produce bioactive substances which effective in restrain the growth of 3 species of bacteria, i.e., *Aeromonas* spp., Enterobacteriaceae, and *Pseudomonas* spp. The species specific bioactives extracts are mostly polar. The non specific species bioactive extracts are semipolar i.e. dissolved in neutral ethyl acetate. Regardless of the species specific property, bioactive sponge extracts are potentially more effective than do antibiotics for fish health management purposes.

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