

THE GROWTH INHIBITION OF MARINE BIOFILM-FORMING BACTERIA BY THE CRUDE EXTRACT OF SOFT CORAL *Sinularia* sp.

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ABSTRACT

Marine biofouling has been recognized as a big problem faced by marine technology, and has caused huge economic losses to marine industries. Until recently, marine biofouling has been controlled by the use of metal-based coating which has become another problem because of their negative impacts on marine environments. Soft corals have been known to produce secondary metabolites, some of which may inhibit the fouling processes.

*The objective of this research was to examine the antimicrobial properties of soft coral *Sinularia* sp against marine biofilm-forming bacteria.*

*The results showed that the soft coral tissues of *Sinularia* sp. had the antibacterial potency. The crude extracts of *Sinularia* sp affected significantly on the growth of bacteria tested. The optimal concentration of crude extracts needed to inhibit the growth of bacteria was 150 µg/ml. There were no significantly different among bacteria isolated from fiber, wood and iron steel on diameter of inhibitory zone of the bacterial growth.*

It is concluded that the search of bioactive substances produced by soft corals is great possibility to find alternatives for metal-based coatings. Yet, a series of researchs must be undertaken in order to find the secondary metabolites which may be used as antifoulant.

Key words : *Sinularia* sp., growth inhibition, crude extract

I. INTRODUCTION

Marine biofouling may be defined as the attachment and metabolism of microorganisms and macroorganisms to solid surfaces submerged in the marine environment (Smith, 1988).

Microbial cells firmly adsorb onto solid surfaces in seawaters and produce extrapolymeres that provide the assembly of primary biofilm. This formation of initial bacterial film is then followed by the attachment of larger organisms, such as, cyanobacteria, diatoms, algae,

protozoa, and the dominantly invertebrate species (Hadfield and Ciereszko, 1978).

Marine biofouling causes huge economic losses to marine industries, since almost all types of structural materials exposed to seawater may become fouled. Haderlie (1984) have listed the problems resulting from the fouling activities including the fouling of ship's hulls, pilings, navigational buoys, underwater equipment, offshore platforms, ocean thermal energy conversion plant, and moored oceanographic instruments. Until recently, marine biofouling has been controlled by the application of heavy metal-based coating paints, which are capable of preventing the structures from fouling activity, however, they are not environmentally acceptable, and caused serious pollution in the marine environments. Miki *et al* (1996) stated that copper and organotin compounds such as tributyltin oxide (TBTO) have been developed as effective antifoulants, however, these metallic compounds are principally toxic. Such compounds have recently been recognized as negatively affecting the marine environment and inducing the malformation of fish (Kon-ya, 1991). It is our responsibility to support the development efforts that promote rapid development of new, non-toxic, environmentally acceptable products that will greatly benefit the marine industry sector in dealing with marine biofouling.

Soft corals have been known to produce secondary metabolites commonly found in their tissues, some of which are unique to one, or a few closely species. The type, concentration, and function of these secondary metabolites vary widely. One of the

most interesting phenomena within soft corals is that they are usually characterized by the absence of fouling organisms on their surfaces. The mechanism by which soft corals maintain their clean surfaces is strongly believed to represent the ecological role of secondary metabolites, some of which may inhibit the fouling process (Colwell, 1988). So, the search of bioactive substances produced by soft corals is a great possibility to find alternatives for metal-based coatings. However, several phases must be carried out in any study, such as, collection of soft corals, extraction, preliminary screening, bioassay-directed isolation and purification of the active compounds, structure elucidation, synthesis, analog production of the active compounds, and field tests, before the secondary metabolites from soft corals are used as alternative marine antifoulants.

The objective of this study is to investigate the antimicrobial properties of soft coral *Sinularia sp.* againsts marine forming-biofilm bacteria isolated from fiber, wood and iron substrates.

II. MATERIALS AND METHODS

2.1. Bacterial Strains

The bacterial strains used in this study are *Sporosarcina sp.*, *Bacillus sp.* and *Camphylobacter sp.* These marine biofilm-forming bacteria were isolated from film layers of iron, wood and fiber substrates (Sabdono *et al*, 1998). Zobell 2216E medium, pH 7.6 at room temperature, was used for maintenance of these strains.

2.2. Sample Collection

Soft corals *Sinularia sp* were collected at the seashore of Pulau Panjang, Jepara. They were kept in a plastic bags filled with seawater and brought to the Marine Station, Teluk Awur, Jepara.

2.3. Preliminary Test

100 μ l suspensions of marine forming-biofilm bacteria was laid over Zobell 2216 agar medium. A piece of tip soft corals, 5 gr in weight, was placed onto agar medium. The plates were then incubated at room temperature for 24 h. After incubation, the formation of growth inhibition was observed.

2.4. Extraction

Extractions were carried out using the procedures described by Montano and Glorioso (1994). Samples were homogenized with solvent system consisting of hexane (non polar) and 10% methanol. Filtrate and solvent were separated and roto-evaporated under vacuum.

2.5. Microbial Assays

Extract was tested for antimicrobial activity using a standard agar

plate assay disk method (McCaffrey and Endean, 1985). 100 μ l suspensions of marine forming-biofilm bacteria was laid over Zobell 2216 agar medium. The agar plate was divided into six equal sections onto which the extract-treated disks were placed. Paper disk (8 mm in diameter) was placed onto agar medium and extract concentration (150, 300, 450, 600 and 750 μ g/ml) was dropped on paper disk allowing diffusion of the extract into the agar. All assays were done in triplicate. After an overnight incubation, zone inhibition were measured.

III. RESULTS AND DISCUSSION

3.1. Antimicrobial Assays

The result of preliminary test showed that soft coral tissues of *Sinularia sp.* had the antibacterial potency (Figure 1). The presence of antibacterial agents in soft corals was supported by the results reported in two earlier studies. Burkholder (1973) and Rinehart *et al* (1981) reported that antimicrobial activity has been demonstrated in numerous marine invertebrate taxa.

The activity of soft coral extracts against the three species of bacteria was demonstrated in Table 1, 2 and 3.

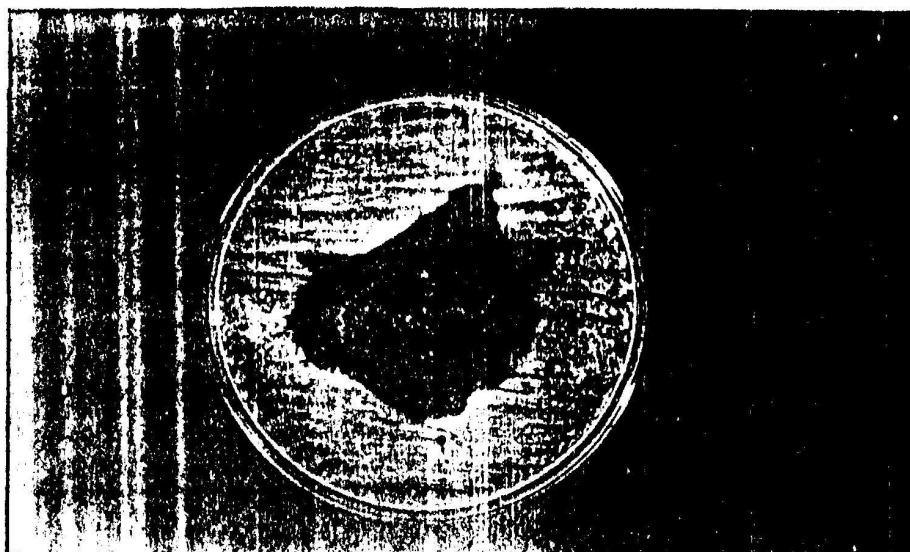


Figure 1.

Preliminary test of soft coral tissues against marine biofilm-forming bacteria

Table 1.

Summary of ANOVA performed on antimicrobial activity

Source of Variance	df	SS	MS	F-ratio	F _{0.05}
Main Plot					
Bacteria	2	4.348	2.174	6.003	6.94
Error (a)	4	1.441	0.360		
Sub-Plot					
Extract Concentration	5	4738.270	947.654	156.586**	2.53
Interaction BxE	10	17.500	1.750	0.289	2.16
Error (b)	30	181.559	6.052		
Total	53	5412.801			

Table 2.

The effect of extract concentration on the formation of inhibition zone

No.	Extract concentration ($\mu\text{g/ml}$)	Inhibition zone (mm)
1.	Kontrol	7.011 a
2.	150	20.244 b
3.	300	26.167 c
4.	450	28.522 cd
5.	600	33.289 d
6.	750	34.889 d

Table 3.

Bacterial sensitivity to extract

No.	Species	Inhibition zone (mm)
1.	<i>Sporosarcina sp.</i>	25.044 a
2.	<i>Bacillus sp.</i>	25.356 a
3.	<i>Camphylobacter</i>	24.661 a

This study represents the quantitative assay for the presence of antimicrobial properties of extracts from soft coral *Sinularia sp.* Extract from soft coral *Sinularia sp.* demonstrated antimicrobial activity. There were no significant difference among marine biofilm-forming bacteria in antimicrobial response to extracts by measuring the diameter of inhibition zone (Table 1 and 3). This result was supported by Amade *et al* (1982) that found an equal number of gram-positive and gram-negative bacteria sensitive to sponge extract. However, the comparative insensitivity of bacteria documented in this study is not consistent with the finding of

McCaffrey and Endean (1985). In this phenomenon, the correlation of antimicrobial activity and response of test microorganism is still unclear since the the diversity of of natural products and bacterial response.

In assaying the natural products from soft coral *Sinularia sp.*, there were significant difference on extract concentration treatments (Table 1 and 2). The higher the extract concentration, the greater the growth inhibition. However, there were no significant differences among 450, 600 and 750 $\mu\text{g/ml}$ extract concentration. The optimal concentration of crude extracts needed to inhibit

the growth of bacteria was 150 µg/ml. It could be concluded that stationary phases to kill those bacteria started at 300 µg/ml extract concentration. Kim (1994) stated that each coral species may possess its own unique array of antimicrobial agents with a specific level of effectiveness against different bacteria. Thus, the antimicrobial properties may be a function of efficacy of a particular agent and not necessarily of concentration.

IV. CONCLUSION

The search of bioactive substances produced by soft corals is great possibility to find alternatives for metal-based coatings. Yet, a series of research must be undertaken in order to find the secondary metabolites which may be used as antifoulant.

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