Biological Activity of Extracts of *Sargassum oligocystum* (Magnaye) against Aquaculture Pathogenic Bacteria

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Abstract

*Sargassum oligocystum* (Phaeophyceae) was collected from the coastal area of Sta. Ana, Cagayan, Philippines, and used in *in vitro* antibacterial assays against six pathogenic bacteria commonly occurring in aquaculture. The extracts (methanol, n-hexane, dichloromethane, ethyl acetate, aqueous) were screened against *Vibrio harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *Flavobacterium aurantiacum*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa*. The methanol extract showed strong antibacterial activity against *V. harveyi*, *S. faecalis*, and *P. aeruginosa* and moderate activity against the rest of the test pathogens. In general, *V. harveyi* was the most susceptible strain to all the extracts. This study suggests that extracts of *S. oligocystum* may be promising sources of antibacterial agents for use in aquaculture.
Introduction

Disease outbreaks are common in the rapidly developing aquaculture industry. Antibiotics, prophylactic chemicals, and chemotherapeutants are used to treat bacterial fish diseases. However, the occurrence of mutants and antibiotic-resistant bacteria have become major problems.

The marine environment is a source of diverse products having structurally novel and biologically active metabolites. Such products can be sources of natural immunostimulants that prevent infectious disease. Natural products of marine macroalgae possess bioactive potential (Hellio et al., 2001; Ely et al., 2004; Selvin and Lipton, 2004). Sponges can inhibit the growth of marine fish pathogenic bacteria (Selvi Sonia et al., 2008). Red (Rhodophyceae), green (Chlorophyceae), and brown (Phaeophyceae) algae possess antimicrobial properties against pathogenic bacteria (Febles et al., 1995; Bansemir et al., 2006; Fareed and Khairy, 2008; Chiheb et al., 2009; Vallinayagam et al., 2009). Macroalgae have inhibitory activity against human pathogenic bacteria, fungi, and yeasts (Sridhar and Vidyavathi, 1991; de Val et al., 2001; Liao et al., 2003; Bansemir et al., 2006; Jean Jose et al., 2008; Lipton et al., 2009). Seaweeds contain high levels of minerals, vitamins, essential amino acids, indigestible carbohydrates, and dietary fibers (Kotnala et al., 2009) and are a rich source of many bioactive and pharmacologically important compounds such as alginate, carageenan, and agar, phycocolloids that are used in medicine and pharmacy (Siddhanta et al., 1997). Bioactive compounds in seaweeds produce secondary metabolites with a wide spectrum of biological activity (Bansemir et al., 2006).

The seaweed Sargassum oligocystum is widely distributed in coastal areas throughout the Philippine archipelago. In this study, we screened the ability of four extracts of the brown algae S. oligocystum collected off the coast of Cagayan, Philippines, to inhibit the growth of six pathogenic bacteria with the aim of possibly using them as alternative antimicrobial agents for aquaculture.

Materials and Methods

Seaweed sampling and preparation of extracts. Seaweeds (Sargassum oligocystum) were collected by scuba diving and handpicking from the rocky substratum at depths of 1-2 m along the subtidal areas of Sta. Ana, Cagayan (18°25′ 25.50″ N, 122°07′28.20″ E), Philippines. The collected seaweeds were cleaned of epiphytes, extraneous matter, and necrotic parts, washed with clean fresh water, air-dried at room temperature for two weeks, cut into small portions, and powdered using a hammer mill and a mechanical food blender. Extracts (methanol, n-hexane, dichloromethane, ethyl acetate) of the powdered seaweed were obtained with organic solvents to increase polarity.

Antibacterial assay. Disk diffusion assays were performed according to Ruangpan and Tendencia (2004). Sterile paper disks (6-mm diameter) were prepared using Whatman no. 1 filter paper autoclaved for 15 min at 121°C and impregnated with 20 µl (500 mg/ml) of the crude extracts. The tested bacterial pathogens were Vibrio harveyi PN-9801 (de la Peña et al., 2001), Vibrio paraaerolyticus BIOTECH 10210, Vibrio alginolyticus BIOTECH-1986, Flavobacterium aurantiacum BIOTECH 1492, Streptococcus faecalis BIOTECH 1072, and Pseudomonas aeruginosa. The bacteria were subcultured in Difco nutrient agar (+ 1.5% NaCl for the marine bacteria) for 24 h prior to use. Each test organism was separately suspended in a 5-ml trypticase soy broth (Difco) solution. Turbidity was compared using McFarland’s solution. The concentration of bacteria in each solution was approximately 1.2 x 10^8 cfu/ml. Mueller-Hinton Agar (MHA) was surface inoculated with this suspension for each organism. The crude seaweed extracts were placed on the MHA medium and the plates were incubated at 32°C for 24 h. Chloramphenicol (500 mg/ml) was used as a positive control and the solvent of each extract as a negative control. The diameters (mm) of the growth inhibition halos caused by the extracts were measured. The antibacterial assay was carried out in triplicate.
Results

Methanol, n-hexane, dichloromethane, and ethyl acetate extracts showed significant inhibitory effects against most of the tested bacteria (Table 1). Vibrio harveyi was the most susceptible organism; its growth was strongly inhibited by the methanol, dichloromethane, and n-hexane extracts and moderately inhibited by the ethyl acetate extract. The strongest antibacterial activity was obtained by the methanol extract, with inhibition zones of 22 mm against V. harveyi, 20 mm against S. faecalis, and 17 mm against P. aeruginosa. Statistical differences ($p<0.05$) between zones of inhibition (means±SE) for each bacteria against each extracts are presented in Fig. 1.

Table 1. Antibacterial activity of Sargassum oligocystum extracts against pathogenic aquaculture bacteria.

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<tr>
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<th>Vibrio harveyi</th>
<th>Vibrio parahaemolyticus</th>
<th>Vibrio alginolyticus</th>
<th>Flavobacterium aurantiacum</th>
<th>Streptococcus faecalis</th>
<th>Pseudomonas aeruginosa</th>
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<td>Sargassum oligocystum extract</td>
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<td>Aqueous</td>
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- = No activity, + = weak (7-10 mm diameter), ++ = moderate (10-15 mm diameter), +++ = strong (>15 mm diameter). Negative controls (solvents) not shown since none exhibited activity.

Discussion

Extracts of S. oligocystum collected from the coastal waters of Cagayan, Philippines, inhibited the growth of most of the studied pathogenic bacteria, similar to brown algae extracts in other studies (Febles et al., 1995; Kandhasamy and Arunachalam, 2008; Patra et al., 2008; Kotnala et al., 2009). Results between studies can vary, depending on the location and season in which the samples are collected, organic solvents used to extract the bioactive compounds, or differences in the assay method (Kandhasamy and Arunachalam, 2008). Differences in the antimicrobial activity of different seaweeds may also be attributed to the multiple inhibitory properties found in seaweeds; phytochemical contents of phenols, tannins, alkaloids, anthraquinones, flavonoids, cardiac glycosides, saponins, steroids, and terpenoids vary among seaweeds from traces to abundance (Kotnala et al., 2009).

Having been shown to possess biologically active compounds, S. oligocystum extracts may have potential as alternative antibacterial agents, replacing commercial antibiotics and chemotherapeutics for prophylaxis and therapy of bacterial fish diseases. Bioactive compounds from S. oligocystum can be characterized and purified, leading to synthetic production of antimicrobial compounds for commercial use. Studies on the nutritional aspects, stability, metabolism, toxicity, and economics of seaweed and seaweed components must be considered.
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References


