Bull. Environ. Contam. Toxicol. (1992) 49:743–749

Environmental Contamination and Toxicology

Production of Antibacterial Substances by Macroalgae of the New York/New Jersey Coast, USA

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Marine algae are known to produce biochemicals which show a high degree of toxicity. The neurotoxin produced by the dinoflagellate Gonyaulax polyedra is responsible for red tides in tropical waters (Atlas 1984). Research has revealed that several of these substances have antibacterial, antiviral and/or antineoplastic effects (Scheuer 1990). Kainic acid, derived from the red algae Digenia simplex is an active antihelminthic (Grant and Mackie 1977). The toxic nature of these substances, which include acrylic acid, phenols, terpenoids and halogenated compounds, has made many of them unfit for commercial exploitation (Burkholder and Sharma 1969). They are produced by the algae to aid in plant protection by acting as deterrents for herbivorous fish (Horn et al.1985) and to prevent surface fouling by bacteria and other epibionts (Norris and Fenical 1982).

These substances may be contained in different parts of the algal thallus. In some species the new areas of growth have higher concentrations, perhaps to better protect these more fragile regions (Hornsey and Hide 1976; Lustigman and Brown 1990). Different phases of the life cycle of the algae are also associated with varying amounts of antibiotic production (Hornsey and Hide 1985).

Previous research has shown production of antibiotic substances by marine macroalgae which were effective in preventing the growth of human clinical bacteria (Lustigman and Brown 1990). Many marine bacteria however, live in or on the algal thallus (Barbeyron and Berger 1989) and may have greater resistance to these substances. In this study we report the effect of antibacterial substances produced by 35 seaweed species as challenged by three species of marine bacteria. A preliminary extraction of the inhibitory substances produced was also undertaken.

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MATERIALS AND METHODS

Samples of 35 seaweed species were collected from Sandy Hook, New Jersey and Montauk Point, New York from March to September, 1989(Table 1). After collection the samples were placed on ice for transport to the laboratory. They were washed in 10% Chlorox, rinsed twice in distilled water and the different species were identified based on the monograph by Hillson (1977). Samples were then frozen until use. Samples of 3 cm were cut from the algal thallus, including the growing tip. These were surface sterilized using 10% Chlorox and rinsed in sterile distilled water. Each piece was then placed in a Petri dish with molten Difco Marine Agar in order to produce uniform contact of the algal sample and the agar. After the agar hardened, each dish was streaked with 0.1 mL from a 6-8 hr bacterial culture of one of the three bacterial species, Vibrio marinofulvis, Micrococcus imfimus, Pseudomonas atlantica. Duplicates of each of the samples were prepared. Controls were prepared using marine agar and cultures of bacteria to determine culture viability. After 18-24 hrs cultures were observed and zones of inhibition were measured. If the zone was not uniform, four measurements from different areas were taken. The mean was calculated and this figure was used.

Raw extracts were prepared from the seaweed samples which had previously demonstrated activity against the marine bacteria. Individual samples were separated into 10 g portions and blended with one of the following solvents: methanol, chloroform or water in a Waring blender for 10 minutes. Samples were centrifuged for 10 minutes at 10Xg. The supernatant was filtered and allowed to evaporate until dryness. The total dry weight was obtained. The extract was resuspended in the appropriate solvent to give a final concentration of 40ug/uL. Sterile filter paper discs were impregnated with 100uL of resuspended extract (4mg) and air dried. Plates containing marine agar were inoculated with one of the three bacterial species. The discs were placed on the bacterial lawn. After 18-24 hours the zones of inhibition were noted and measured.

RESULTS AND DISCUSSION

The data show that substances are produced by seaweeds of the New York/New Jersey coast, which inhibit the growth of marine bacteria, at least to some extent (Table 1). Pseudomonas atlantica was not susceptible to these substances. Vibrio marinofluvus showed limited inhibition, a zone of inhibition from 3 seaweed species. A greater effect was seen with Micrococcus imfimus; inhibition occurred with 8 species. While the first two species are gram negative, the last is gram positive. Gram positive bacteria are known to be more sensitive to certain antibiotics, due to differences in cell wall structure. The gram negative bacteria are

Table 1. Zones of inhibition of bacteria

Seaweed	Zones of inhibition (mm)		
	Vibrio (Gram -)	Micro (Gram+)	Pseudo (Gram -)
CHLOROPHYTA			
Chaetomorpha linum	6	0	0
Cladophora gracilis	0	3.5	0
Codium fragile	0	0	0
Codium isthmocladium	0	5	0
Enteromorpha linza	0	7.5	0
Spongomorpha lanosa	7.5	0	0
Ulva lactuca	0	0	0
PHAEOPHYTA			
Ascophyllum nodosum	0	0	0
Chordaria flagelliformis	0	0	0
Dictyosiphon foeniculaceus	0	0	0
Ectocarpus tomentosus	0	0	0
Fucus edentatus	0	4.5	0
Fucus evanescens	0	0	0
Fucus filiformis	0	0	0
Fucus serratus	3	0	0
Fucus spiralis	0	4.25	0
Fucus vesiculosus	0	4	0
Laminaria agardhii	0	3	0
Laminaria saccharina	0	0	0
Pilayella littoralis	0	0	0
RHODOPHYTA			
Ahnfeltia plicata	0	0	0
Ceramium rubrum	0	0	0
Chondrus crispus	0	0	0
Cystoclonium purpureum	0	0	0
Gracilaria verrucosa	0	0	0
Grinnellia americana	0	0	0
Laurencia sp	0	0	0
Phyllophora brodiaei	0	0	0
Polysiphonia denudata	0	0	0
Polysiphonia fibrillosa	0	0	0
Polysiphonia harveyi	0	0	0
Polysiponia lanosa	0	5	0
Porphyra leucosticta	0	0	0
Porphyra umbilicalis	0	0	0
Rhodymenia palmata	0	0	0

a
Vibrio = Vibrio marinofulvis
Micro = Micrococcus imfimus
Pseudo = Pseudomonas atlantica

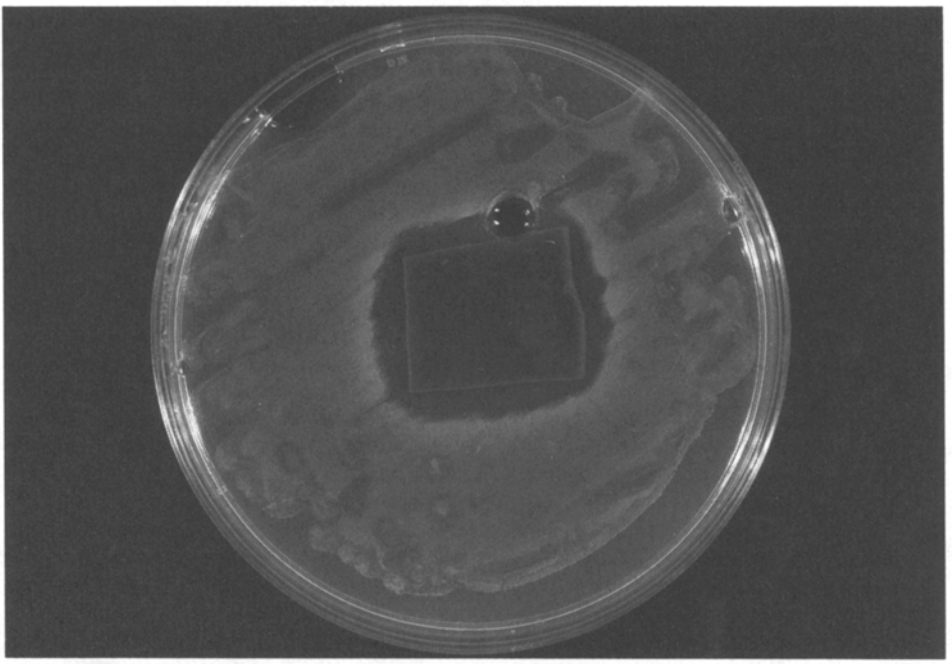

Figure 1. Zone of inhibition produced by Laminaria agardhii against Micrococcus imfimus.

surrounded by a complex cellular envelope and therefore have a greater resistance to many antibiotics. Among the seaweeds, the chlorophyta displayed the most activity, 4 of 7 species (57%). The phaeophyta showed activity in 5 of 13 species (38%), mainly among the Fucales. The rhodophyta seem to be the least effective in the production of these substances, with only one active species. Not all species within a genus showed activity. Among the six Fucus species tested most were active, except for F. evanescenus and F. filiformis.

Comparison of these results with previous work (Lustigman and Brown,1990), shows that human clinical bacteria displayed far greater susceptibility to the substances produced by the algae than did the marine species. With marine bacteria, the zones of inhibition were much smaller and the number of seaweeds producing biologically active substances was fewer. Marine bacteria, which might have been exposed to these substances in the natural environment, would be more likely to have developed resistance to them than would clinical bacteria.

Table 2. Zones of inhibition using algal extracts

Seaweed	Zones of inhibition(mm)								
	Vibrio			Micro			Pseudo		
	M	C	W	M	C	W	M	C	W
Chordaria flagelliformis	0	0	0	0	0	0	0	0	0
Codium isthmocladium	0	0	0	0	0	0	0	0	0
Fucus endentatus	0	0	0	19	0	19	0	0	0
Fucus serratus	0	15	0	0	0	0	0	16	0
Fucus spiralis	0	0	0	20	15	20	0	0	0
Fucus vesiculosis	0	0	0	0	0	0	0	0	0
Laminaria agardhii	0	0	0	20	19	20	0	0	0
Spongomorpha lanosa	0	0	0	0	0	0	0	0	0

a Vibrio = Vibrio marinofulvis
Micro = Micrococcus imfimus
Pseudo = Pseudomonas atlantica

b M = methanol
C = chloroform
W = water

Table 2 gives the data obtained for the extracts prepared from those algae which showed promise of bactericidal activity. The extracts were prepared using either methanol, chloroform or water as solvents. The results indicate that for half of the seaweeds tested, at least one of the extracts was active. The zones of inhibition were also somewhat larger than with the seaweed thallus samples, probably due to greater concentration of the substances in the discs.

Different antimicrobial substances are produced by the seaweeds, as is seen by the difference in activity dependent upon the solvent used. Micrococcus was more susceptible than the gram negative species, although the extract of F.serratus in chloroform displayed activity against Vibrio and Pseudomonas,but not Micrococcus. The Fucales were the most active of the seaweeds tested. Results were similar to those achieved with human clinical bacteria, with the clinical bacteria again more susceptible to the antibiotic substances (Lustigman and Brown 1990).

Most of the members of the Fucales displayed bactericidal activity. Polyphenols are known to be excreted by phaeophyta, such as F.spiralis (Bhakhuni and Silva 1974). These chemicals cause cell lysis through membrane damage. Terpenoids produced by the phaeophyta have also shown to be antimicrobial and ichthyotoxic (Schlenk and Gerwick 1987; Fenical 1982). The results for the chlorophyta are similar to those achieved in prior reports, with activity seen with the Enteromorpha linza and Spongomorpha kutzung (Lustigman and Brown, 1990). Chlorophyllides are among the active substances isolated from chlorophytes and phaeophytes (Burkholder and Sharma 1969). Terpenoids are also

produced by chlorophyta and rhodophyta (Fenical 1982). Acrylic acid is widely produced by the green, brown and red algae (Burkholder and Sharma, 1969). Many of the compounds produced are brominated, causing them to be highly toxic (Caccamese et al. 1981). Further research is underway to determine the chemical nature of the substances produced by the seaweeds studied here.

Substances produced by macroalgae of the New York/New Jersey coast can be toxic to marine bacteria. There is a high survival value for algae producing secondary metabolites that are detrimental to organisms susceptible to those chemicals (Scheuer 1990). Marine bacteria showed more resistance to these substances than bacteria normally associated with humans, since the former have adapted over the generations to the presence of such substances produced and released in seawater.

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Received Received January 13, 1992; accepted April 30, 1992.