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Antibiotic Production by Marine Algae Isolated from the New York/New Jersey Coast

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Marine algae have long been known to produce toxic substances. *Gonyaulax polyedra*, the organism responsible for Florida red tides, produces a highly toxic neurotoxin (Atlas 1984). Research has revealed the production of antimicrobial, antiviral and other substances by marine macroalgae (Ratafia and Purinton 1988). Many of these substances have been shown to be too toxic for pharmaceutical purposes. Nielson (1955) emphasized the role of antibiotics produced by marine algae in controlling the growth of marine bacteria. Certain marine algal species are free of, or have seasonality with regard to, epiphytic growth (Sieburth and Tootle, 1981).

Hornsey and Hide (1976a,1976b) examined 151 species of British algae. They determined species activity, seasonal variation, and selective concentration within the algal thallus. Caccamese et al. (1980,1981) studied the effect of extracts from algae of the Mediterranean against four microorganisms. The algae of Florida have been examined for antimicrobial activity (Hodgson 1974). Fenical (1982) reviewed the chemical nature of several marine antimicrobial products. These secondary metabolites include acrylic acid, terpenes, chlorophyllides, phenols and heterocyclic carbons.

The objective of this research was to undertake a screening of macroalgae from the New York/New Jersey coast for the production of antimicrobials.

MATERIALS AND METHODS

Samples of 39 seaweed species were collected monthly from March to September 1988, at three locations: two in Sandy Hook, N.J. and one at Montauk, L.I., N.Y. After collection the samples were placed on ice

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for transport to the laboratory. There they were washed in 10% Chlorox, rinsed twice in distilled water, and kept frozen in plastic bags until used. Identification was made from two monographs (Taylor 1966, Hillson 1977). Samples of 3 cm diameter 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 Nutrient Agar. This insured uniform contact of the algal sample and the agar. Each dish was streaked with 0.1 ml from a 6-8 hour bacterial culture of either *Escherichia coli* or *Staphylococcus epidermidis*. Controls were prepared to determine bacterial viability. Each algal species was screened in duplicate and incubated at 37^{o}C for 18-24 hours. Algae that produced antibacterial substances exhibited a distinct zone of bacterial growth inhibition around the thallus or growing tip that was measured. If the zone was not distinct, or if it was not relatively uniform, four measurements from different areas were taken. The mean was calculated and this figure was used as the final measurement.

Extracts were prepared from six algal species which had produced positive results in the previous experiments. Ten mg samples (wet weight) from *Chondrus crispus, Chordaria flagelliformis, Enteromorpha linza, Fucus endentatus, F. spiralis* and *F. vesiculosus* were used. The samples were homogenized in a Waring blender with 100 ml of each one of the following extractants: methanol, water or chloroform. The material was centrifuged for 10 minutes at 10G and then filtered through Whatman #1 filter paper. The extracts were evaporated to dryness and weighed. Dry weight extracts were reconstituted in the same solvent to give a final concentration of 4mg/ml. Sterile filter paper disks (1 cm diameter) were impregnated with the reconstituted extracts (100ul). The disks were kept at room temperature until thoroughly dry. Control disks were prepared using only the solvent. The dried discs were placed on Petri dishes containing Nutrient Agar that were inoculated with either *E. coli* or *S. epidermidis*. The dishes were incubated as above. Duplicates of each set were prepared.

RESULTS AND DISCUSSION

Table 1 summarizes the activity of the algal samples. The results show that of the 39 species screened, 18 (46%) exhibited activity against *E. coli,S. epidermidis* or both. Activity was exhibited within all three divisions: 4 of 16 Chlorophyta (25%), 6 of 13 Rhodophyta (46%) and 9 of 11 Phaeophyta (82%). The greatest activity, 16 species, was against gram (+) *S.*

Table 1. Zones of inhibition (mm).

SPECIES	E. COLI	S. EPIDERMIDIS
CHLOROPHYTA		
Cladophorales		
Cladophora gracilis	15.75	18.5
Spongomorpha lanosa	0	5.8
Siphonales		
Codium fragile	0	7.5
Codium dichotomum	0	0
Ulotrichales		
Enteromorpha linza	11.5	11.0
Ulva latuca	0	0
PHAEOPHYTA		
Chordariales		
Chordaria flagelliformis	16.0	28.0
Ectocarpales		
Ectocarpus tomentosus	0	0
Pilayella litoralis	5.0	0
Fucales		
Ascophyllum nodosum	0	10.0
Fucus endentatus	0	8.0
Fucus evanescenus	0	10.0
Fucus serratus	0	15.5
Fucus spiralis	0	total clearance
Fucus vesiculosis	0	8.6
Laminariales		
Laminaria agardhii	0	0
RHODOPHYTA		
Laurencia sp.	3.0	0
Bangiales		
Porphyra leucostica	0	0
Ceramiales		
Ceramium rubrum	0	0
Grinnellia americana	14.0(tip)	7.0(tip)
Polysiphonia denudata	0	0
Polysiphonia fibrillosa	10.5	7.5
Gigartinales		
Agardhiella tenera	0	0
Chondrus crispus	0	6.8
Cystoclonium purpureum	0	8.2
Gracilaria verrucosa	0	0
Rhodymeniales		
Lomentaria sp	0	0
Rhodymenia palmata	0	0

epidermidis (41%). Eight species were active against gram (-) *E. coli* (21%). This is probably due to structural differences between the two types of bacteria. The gram (-) cell wall is more complex, thereby decreasing susceptibility to antimicrobial substances. There were 6 species that exhibited activity against both types of bacteria (15%)--*Cladophora gracilis, Enteromorpha linza, Chordaria flagelliformis, Grinnellia americana, Polysiphonia fibrillosa* and *Agardhiella tenera*.

Not all species within an order or genus showed equal activity. *Codium fragile* was active, but not the closely related *C. dichotomum*. This was also seen with *Polysiphonia fibrillosa* which displayed bioactivity, while *P. denudata* did not.

Distribution of the substances within the thallus varied from species to species. Activity was evenly distributed throughout in *C. flagelliformis* while *G. americana* showed differences of concentration in the older regions compared to the growing tip.

Table 2 gives the results of the extracts prepared from algae selected from among those which showed promise of activity. The extracts were prepared using water, methanol, or chloroform as solvents. Positive results were produced with at least one of the solvents against *S. epidermidis*, with all 6 selected seaweeds. No activity was seen against *E. coli*. Different antimicrobial substances are produced by the seaweeds, as solvent activity varied among the species. *F. spiralis* showed activity in water and methanol, while *F. vesiculosis* was active in methanol and chloroform.

Among the Phaeophyta, the Fucales showed a high level of activity against *S. epidermidis*, which was consistent with the results of previous authors (Henriques et al. 1979; Caccamese and Azzolina 1980). *F. spiralis* produced the best results, showing total plate clearance. Polyphenols are excreted by the Phaeophyta such as *F. spiralis* (Bhakhuni and Silva 1974). These are thought to damage the cell membrane causing eventual cell lysis. *Chordaria*, which clearly produced a substance active against both types of bacteria was reported to be inactive by Hornsey and Hide (1976a). These differences in results may be due to seasonal or regional variations.

Activity can be found in several orders of the Chlorophyta, including species in the Cladophorales, Siphonales and Ulotrichales. Unlike Hornsey and Hide (1976a), we found no antibiotic substance produced by *Ulva lactuca* (Ulotrichales). The Chlorophyta produce

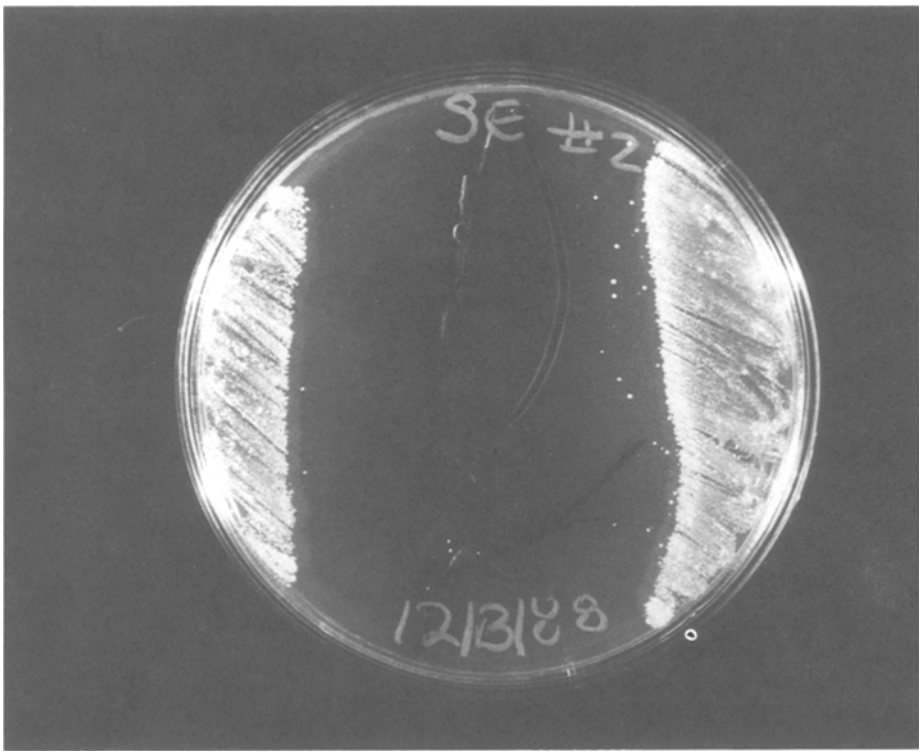

Figure 1. Zone of inhibition produced by Chordaria *flagelliformis* against *S. epidermidis*.

Table 2. Zones of inhibition using solvent extracts against *S. epidermidis*.

Species	Water	Methanol	Chloroform
Chondrus crispus	0	0	16
Chordaria flagelliformis	0	21	0
Enteromorpha linza	0	23	0
Fucus endentatus	24	25	0
Fucus spiralis	27	22	0
Fucus vesiculosis	0	17	23

chlorophyllides which are water-soluble chlorophyll derivatives with antibiotic activity (Burkholder and Sharma,1969). Biological activity of green algae has also been attributed to terpenes (Fenical 1982). Acrylic acid is a common antibacterial of red, green

and brown seaweeds. It has been isolated from extracts of *Ulva* and *Enteromorpha* (Burkholder and Sharma 1969).

In the Rhodophyta, substances effective against *S. epidermidis* were produced by *Chondrus crispus* and *Cystoclonium purpureum*. Positive results for *C. crispus* were previously reported by Hornsey and Hide (1976b). This antimicrobial substance is soluble in chloroform. *Grinellia americana* displayed activity which was limited only to the growing tip. Rhodophyta are known to produce several active substances including acrylic acid and terpenes (Fenical 1982). Bromine has been shown to be incorporated into many of these marine terpenoids which would make them highly toxic (Caccamese et al 1981).

The function of these secondary metabolites is to some degree ecological. Interactions between marine plants and animals are manifested in aquatic population succession and community structure. Their release and activity result in enhanced survival of a species (Norris and Fenical 1982). The concept of biological balance by secondary metabolites has been related to plant protection (Burkholder and Sharma 1969). Antibiotic substances released by macroalgae cause significant changes in their relationships to those organisms susceptible to them, so that their chemical protection value is high as a survival mechanism (Scheuer 1990).

This study has shown that the production of antimicrobials by macroalgae is a regular occurrence among those found in the New York/New Jersey coastal region. Further research is underway to determine the structure and nature of these compounds.

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