Antimicrobial Activity of Marine Algae

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Abstract

It is estimated that 30% of the population of industrial nations suffers annually from foodborne illnesses causing a 35-billion dollar loss in the United States each year. Additionally, the World Health Organization (WHO) reported that nearly 1.8 million people died worldwide in 2005 as a result of diarrheal diseases caused by food contamination. Therefore, finding non-toxic, antimicrobial substances for food preservation has become a priority reflected in the WHO's decision to make food safety one of its top 10 problems to address. One area many researchers have turned to for answers is marine algae due to the vast variety and abundance to be studied. Our purpose was to assess the antimicrobial activity of marine algae. We screened acetonic, methanolic, ethanolic, and aqueous extracts of multicellular marine algae from the central California coast for activity against foodborne pathogens and food-spoilage organisms. The agar diffusion assay was used to test 0.5 g/mL extracts against *Escherichia coli*, *Mycobacterium phlei*, *Aspergillus niger*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, and *Penicillium notatum*. The methanolic and acetonic extracts of a Rhodophyta, *Corallina* sp., inhibited *St. aureus* and *E. coli* indicating the need for further testing. The active compound is being isolated and identified then minimum inhibitory and minimum lethal concentrations will be determined. Initial results suggest the potential for marine algae as a source of effective controls of multiple foodborne contaminants.

Aim

To investigate the antimicrobial properties of various Chlorophyta, Rhodophyta, and Phaeophyta species for use in food preservation.

Background

- Annually, foodborne illness results in millions of deaths and high economic tolls and remains one of the World Health Organization's top priorities (2).
- Foodborne pathogens cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (6).
- There is a need for natural antimicrobials including bacteriophages, bacteriocins, and phytochemical to preserve food (6).
- Macroalgae are good candidates for study because they lack cuticles and should need another defense against pathogens (3).
- Most macroalgae are non-toxic and many cultures have used macroalgae both for their nutritive and medicinal qualities (7).
- About 80% of the world population depends on plant-derived medicines (4).

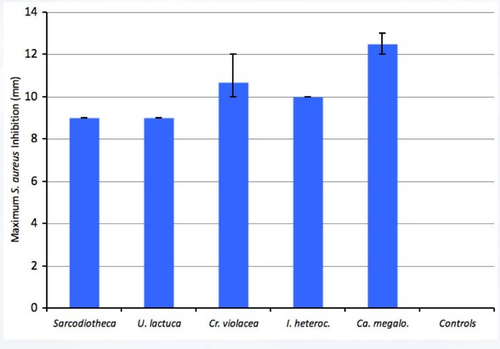


Figure 1. Inhibition of *S. aureus* by acetonic, ethanolic, and methanolic extracts of select algae species. High-low bars represent the minimum and maximum zones where multiple extracts or assays showed inhibition.

Methods

Macroalgae were collected along the central California coast (**Table 1**).

Extract preparation

Sterile aqueous, acetonic, methanolic, and ethanolic extracts were prepared of each alga:

- 1 g algal frond was ground with a mortar and pestle in 2 mL solvent for 3 to 5 minutes.
- Each mixture was placed in a 37°C shaking water bath for 12 to 18 hours.
- The supernatant was transferred to a sterile tube and stored in the dark at 5°C.

Well diffusion assay

- Nutrient agar plates were inoculated with 48- to 96-hr cultures of:
  - *Escherichia coli* (ATCC 11775)
  - *Pseudomonas aeruginosa* (ATCC 10145)
  - *Staphylococcus aureus* (ATCC 27659)
  - Sabouraud dextrose agar plates were inoculated with:
- Sabouraud dextrose agar plates were inoculated with:
  - Spore suspension of *Aspergillus niger* (ATCC 16404)
  - Spore suspension of *Penicillium notatum* (Wards 85w6176)
  - 48-hr culture of *Saccharomyces cerevisiae* (ATCC 9763)
- Tryptic soy agar was inoculated with a 96- to 120-hr culture of *Mycobacterium phlei* (Wards 85W1691).
- 7-mm wells in the agar were filled with 70 µL of extract or solvent (control).
- Bacterial cultures were incubated at 35°C for 48-72 hr. Fungi were incubated at 25°C for 72 to 96 hours.

Results

- *S. gaudichaudii*, *U. lactuca, Cr. violacea, I. heterocarpum,* and *Ca. megalocarpa* inhibited *St. aureus* (**Figure 1**).
- *Cr. violacea* shows the widest range of antimicrobial efficacy, including the only fungal inhibition (**Figure 2**).
- Several species of algae inhibited mycobacteria (**Figure 3**).
- With the exception of *R. parvum*, all species tested showed some microbial inhibition.
- *Cr. violacea* inhibited *A. niger* (**Figure 4**).

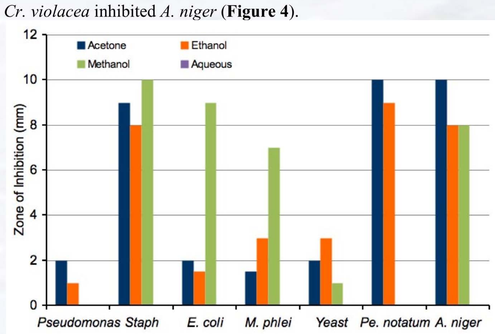
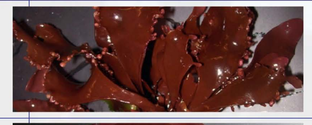
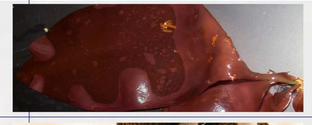
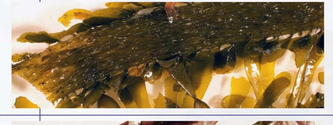
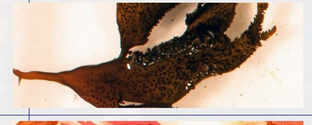
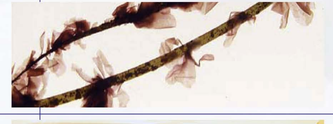
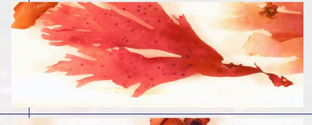


Figure 2. Inhibition of selected microbes by *Cr. violacea*. Values are corrected for inhibition by solvent controls.

Table 1: List of species used in extract preparations.

Postelsia palmaeformis		***Cryptopleura violacea***	
Sarcodiotheca gaudichaudii		***Rhodoglossum parvum***	
Ulva lactuca		***Gigartina papillata***	
Egregia menziese		***Iridophycus heterocarpum***	
Porphyra naiadam		***Callophyllis megalocarpa***	
Nereocystis luetkeana		***Botryoglossum farlowianum***	

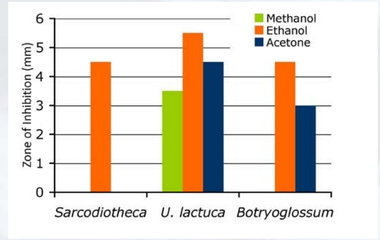


Figure 3. Inhibition of *M. phlei* by algal extracts. Values corrected for inhibition by controls.

Figure 4. This well diffusion plate shows inhibition of *A. niger* by *Cr. violacea* extracts.
M=methanol extract
A=acetone extract
E=ethanol extract
SW= sterile water

Discussion & Conclusion

- Inhibition of *A. niger* by *Cr. violacea* indicates the alga's potential for preventing food spoilage due to molds.
- *Mycobacterium*-inhibition by the algae is promising because of the general difficulty killing multi-drug resistant *M. tuberculosis*.
- *St. aureus* inhibition offers a solution to preventing food intoxication due to growth of *St. aureus* in food. Morover algae may provide a treatment for methicillin-resistant *St. aureus* (1).
- Overall, algae appear to be a viable source for antimicrobial compounds and warrant further testing to isolate, identify, and purify the active substances.

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