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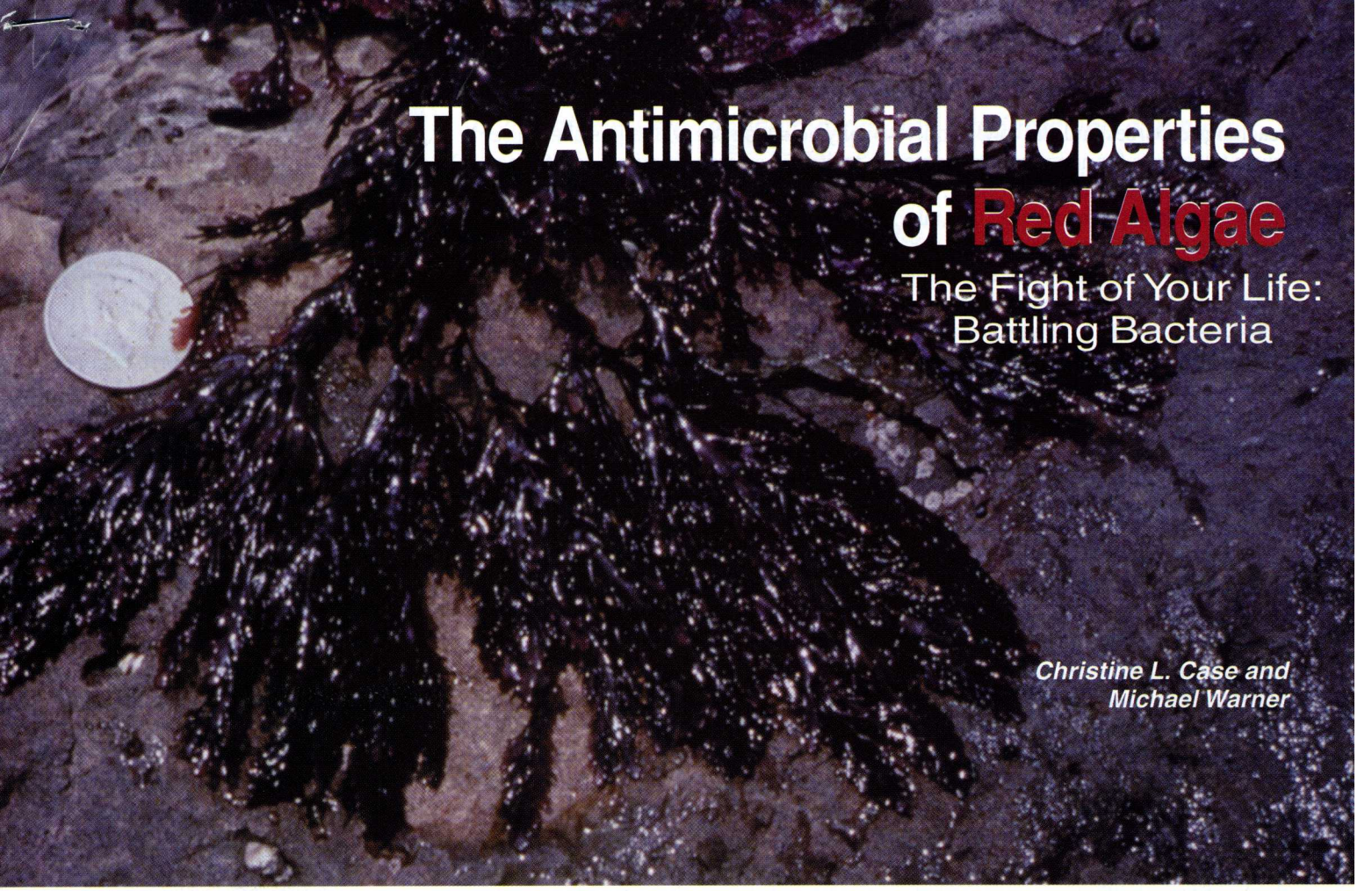

The Antimicrobial Properties of Red Algae

The Fight of Your Life: Battling Bacteria

Christine L. Case and Michael Warner

Undergraduate research gives students the chance to put biological principles into practice and instructors the chance to supervise research close to their interests and expertise. This article describes a research project in which a professor and student collaborated in the screening of macroscopic algae for antimicrobial properties.

A research project provides an ideal opportunity for students to practice scientific techniques and develop their problem-solving skills. Most students majoring in the biological sciences never encounter research opportunities, yet most students want this experience (Carter 1990).

According to the National Research Council and National Science Foundation, an undergraduate science education should prepare students to become both science-literate citizens and competent science professionals (Fort 1995). To make an informed decision to pursue a career in science, students must experience the doing of science, not just the learning of facts. In putting into practice what they learn in theory, students must have training in making deductions and observations, using process skills, and thinking critically (Boud 1980). While these skills are essential to a career in science (Price 1997), too few lab exercises sufficiently challenge the students' ability to reason at higher levels (Sundberg 1991).

In undergraduate research programs, however, students can complete original research projects while working closely with trained experts: the professors themselves (Russo 1997). Barna and Winstead (1993) believe instructors benefit from the collaborative endeavor by acting as a role model for students and, a more practical consideration, maintaining their lab skills, in the end becoming better-trained scientists.

At Skyline College in San Bruno, CA, sophomores majoring in biological sciences conduct their own research projects, reviewing literature and designing and conducting laboratory experimentation, under the guidance of a faculty member. The research program is designed to (1) actively engage students in their learning, (2) foster independent thinking, and (3) encourage self–directed study. A few students have presented their research project findings at local scientific meetings. Here, we describe one of the research projects completed in the course.

Background

With Fleming's discovery of penicillin in the late 1920s, the golden age of antibiotics began. Antibiotics have been

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considered miracle drugs because of their ability to treat bacterial infections without harming healthy human cells. During the last half-century, however, bacteria have developed resistance to many antibiotics. There are some strains of bacteria, including *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Mycobacterium tuberculosis*, for which there are no effective antibiotics.

Ironically, antibiotics themselves promote resistance. Through natural selection, the most fit bacteria, that is, the antibiotic-resistance ones, will survive and pass on resistance traits. Levy (1998) states that antibiotics are misprescribed more than 50 percent of the time, resulting in a grave overreliance on antibiotics. The repercussions of this are evidenced by the number of people who have died in recent years from systemic bacterial infections that did not respond to antibiotics. The solution to this quandary is multidimensional, requiring more responsible use of antibiotics and the development of novel antibiotic compounds.

Since 1942, when Pratt (Lustigman 1988) discovered the antibacterial activity of chlorellin from the green algae, *Chlorella*, a number of researchers have looked to the sea for antimicrobics. Algae from the coastal waters of Great Britain, France, Italy, Brazil, South Africa, India, United States, Saudi Arabia, the Philippines, and Australia, among others, have been sampled for antibacterial properties (Konig 1994, 1997; Mahasneh 1995; Vlachos 1996).

In this study, we (professor and student) worked together to screen macroscopic algae for antimicrobial properties against selected gram-positive and gram-negative bacteria. We used the agar-diffusion method, a well-established techniques for testing natural sources for antimicrobial properties, to screen algal pieces and algal extracts (Crueger 1989; Krieg 1994).

Materials and Methods

We collected the algae listed in table 1 from several sites in the Monterey Bay National Marine Sanctuary and the Gulf of the Farralones National Marine Sanctuary. The algae were removed from the intertidal zone and frozen at -5°C within 12 hours of collection. We obtained *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacteria from the Skyline College Culture Collection.

Nutrient agar plates were inoculated with 100 µL of a 24-hour bacterial culture. Within 24 hours of collection, we rinsed the algal samples with sterile water and placed one-cm pieces on the inoculated plates for an agar diffusion assay. The plates were then incubated for 24 hours and checked for zones of inhibition. We ran controls with 10 µg disks of ampicillin against the gram-positive bacteria and 30 µg disks of tetracycline against the gram-negative bacteria. If we observed an initial zone of inhibition then we incubated the plates for an additional 24 hours to

Table 1. *Bacterial inhibition of selected bacteria by algal pieces in an agar diffusion assay.*

\+ with no diameter ≤ 2mm zone of inhibition; ND = not done

	Presence of zone of inhibition against		
Algae	***S. aureus***	***E. coli***	***P. aeruginosa***
Osmundea spectabilis	+ (6 mm diameter zone of inhibition)	+ (8 mm diameter zone of inhibition)	+ (14 mm diameter zone of inhibition)
Callithamnion pikeanum	-	-	-
Mazzaella flaccida	-	-	-
Mastocarpus pappillata	-	-	-
Neorhodomela larix	+	+	+
Odonthalia floccosa	+	+	+
Mazzaella heterocarpa	-	-	-
Porphyra perforata	+	-	-
Pelvetiopsis limitata	-	-	-
Fucus gardneri	+	-	-
Tetracycline, 30 µg	ND	+ (26 mm diameter zone of inhibition)	+ (9.5 mm diameter zone of inhibition)
Ampicillin, 10 µg	+ (18 mm diameter zone of inhibition)	ND	ND

Selected original research conducted by Skyline College students and presented at scientific meetings, 1995–1998.

- Development of Metal Tolerance in Bacteria in Drinking Water Distribution Systems.
- Novel Antimicrobial Agents Against *Staphylococcus aureus.*
- Antimicrobial Properties of Juglone, a walnut metabolite.
- The Prevalence of Antibiotic Resistance in Deep Subsurface Bacteria.
- Development of Resistance in *Enterococcus faecalis* Exposed to Ampicillin or Garlic.
- A Biological Method to Test the Effectiveness of Sunscreens.
- Evaluation of Juglone to Prevent Post–Harvest Decay.
- Identification of Deep Subsurface Bacteria.

evaluate whether resistant organisms would grow.

Finally, we rinsed samples of *Osmundea spectabilis* (approximately 18–20 cm in size with blades that are flat and pinnate) with sterile water and mechanically ground the samples with a mortar and pestle in each of the following solvents: methanol, ethanol, and methyl chloride.

Results and Discussion

Most algae demonstrated minimal or no zone of inhibition in the agar diffusion assay (table 1). A few algae, however, especially *O. spectabilis*, did cause a zone of inhibition. Of particular note was the fact that, after 24 hours, *P. aeruginosa* began to grow in the tetracycline zone of inhibition but not in the *O. spectabilis* zone of inhibition.

We used agar-diffusion on nutrient agar plates with *P. aeruginosa* to test extracts of *O. spectabilis,* looking for an appropriate solvent for the antimicrobial metabolite(s). We then tested extracts of *O. spectabilis* to find a solvent for extracting the active compound. We observed no zones of inhibition from the methanol and ethanol extracts of *O. spectabilis,* although the methyl chloride extract produced a 1.8-cm zone of inhibition against *P. aeruginosa*. Methyl chloride alone did not inhibit growth of *P. aeruginosa.*

After performing this study, we discovered that methyl chloride extracts containing the active ingredients in the red alga, *O. spectabilis*, have the potential to treat bacterial infections, especially against *P. aeruginosa.* Antibacterial properties have been observed in *Osmundea* spp., which are found in warmer waters (Konig 1994, 1997); *O. spectabilis,* however, is found in cold water throughout the intertidal zones along the California coast. At present, we are characterizing the active metabolites in *O. spectabilis*.

This type of research, besides being topical and relevant, offers students experience in the lab. Russo (1997) states that students need to repeat lab techniques to master them as well as verify results and eliminate errors. Research projects give students just this experience.

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