

# PRELIMINARY SURVEY OF FUNGISTATIC PROPERTIES OF MARINE ALGAE

ANN MARIE WELCH

*United States Veterans Administration Hospital, Durham, North Carolina*

Received for publication July 10, 1961

## ABSTRACT

WELCH, ANN MARIE (U. S. Veterans Administration Hospital, Durham, N. C.). Preliminary survey of fungistatic properties of marine algae. *J. Bacteriol.* **83**:97-99. 1962—Homogenized preparations of 35 marine algae were tested for inhibitory activity against 6 pathogenic or opportunistically pathogenic fungi with saturated filter-paper discs on seeded Sabouraud agar plates; 11 of these preparations produced wide zones of inhibition against 1 or more test organisms, and at least 4 of the 11 are considered to be worthy of further study. The results indicated that further search should be made for antifungal substances from marine algae.

Antibiotic substances from marine organisms have been studied by numerous workers (Pratt et al., 1944; Vacca and Walsh, 1954; Chesters and Stott, 1956; Allen and Dawson, 1960), but, heretofore, the pathogenic fungi have not been used as test organisms except on two occasions: recently, when *Candida albicans* was included with a number of bacteria which were tested against Puerto Rican algae (Burkholder, Burkholder, and Almodovar, 1960); and, several years ago, when *Trychophyton mentagrophytes* and *T. rubrum* were used in assays of extracts from seaweeds of the coast of California (Pratt et al., 1951; Mautner, Gardner, and Pratt, 1953). Therefore, when an opportunity arose to visit the field station of the Institute of Marine Biology of Puerto Rico, it was considered of interest to extend the previous work on antibiotic properties of Puerto Rican algae (Burkholder et al., 1960), and to determine if the same or different algae may have antifungal properties when tested against a representative group of pathogenic and opportunistically pathogenic fungi.

## MATERIALS AND METHODS

The algae were collected at depths of 30 feet or less, primarily from coral reefs and mangrove

islands, by wading or diving. They were placed in separate bottles in the field, and brought into the laboratory within 2 to 3 hr after collection. Voucher specimens were prepared and pressed, and the major portion of each sample numbered and frozen. For assays, samples were thawed and portions of 2 to 3 g were rinsed briefly in distilled water and ground with mortar and pestle into a paste, which was applied on filter-paper discs to seeded plates of the test organisms on Sabouraud dextrose agar and incubated at room temperature. A total of 68 homogenized preparations, representing 35 distinct species, were tested against six fungi. The test organisms used were: *Aspergillus niger*, *Penicillium* sp., *Mucor racemosus*, *Rhizopus oryzae*, *Candida albicans*, and *Cryptococcus neoformans*. It had been planned to use a dermatophyte and the pathogens *Blastomyces dermatitidis* and *Histoplasma capsulatum*, but, since it was desired to test the preparations without autoclaving or Seitz filtration, the plates rapidly became contaminated with marine bacteria and yeasts. Consequently, it was not feasible to use as test organisms those which could not be read within 36 to 42 hr after plating. The zones of inhibition were measured from the edge of the paper disc to the point of cessation of growth.

## RESULTS

The results are shown in Table 1. In some cases an alga was collected and tested more than once. In these instances, the greatest zones of inhibition are the ones recorded. The numbers refer to the width, in millimeters, of the zone of inhibition, or, if a number is preceded by "S," it refers to the width of the zone of stimulation. In some cases an alga both inhibited and stimulated growth, a zone of inhibition being surrounded by an area of increased growth of the test organism. These instances are indicated by a double entry. The symbol "W" refers to large zones, of 15 mm or more, on the *Rhizopus* plates where both the hyphae and the sporangia were completely white. These zones were very striking in their contrast

TABLE 1. Inhibitory activity of marine algae against pathogenic or opportunistically pathogenic fungi\*

| Algae assayed                               | Assay organisms        |                        |                          |                        |                         |                                |
|---|------------------------|------------------------|--------------------------|------------------------|-------------------------|--------------------------------|
|   | <i>Rhizopus oryzae</i> | <i>Mucor racemosus</i> | <i>Aspergillus niger</i> | <i>Penicillium</i> sp. | <i>Candida albicans</i> | <i>Cryptococcus neoformans</i> |
| Rhodophyta (red algae)                      |                        |                        |                          |                        |                         |                                |
| <i>Amphiroa fragilissima</i>                | I-3, W                 | I-1                    | I-5                      | I-4                    | 0                       | I-1, S                         |
| <i>Ceramium nitens</i>                      | 0                      | 0                      | 0                        | 0                      | 0                       | S-8                            |
| <i>Coelothrix irregularis</i>               | W                      | 0                      | 0                        | I-2, SF                | 0                       | 0                              |
| <i>Hormothamnion entermorphoides</i>        | I-3, W, PF             | I-2                    | I-5, PF                  | S                      | I-2                     | I-2                            |
| <i>Hypnea cervicornis</i>                   | 0                      | 0                      | 0                        | I-2                    | 0                       | S                              |
| <i>Laurencia obtusa</i>                     | I-8                    | I-6                    | I-5                      | I-5                    | 0                       | I-2                            |
| <i>L. papillosa</i>                         | 0                      | 0                      | 0                        | 0                      | 0                       | I-12                           |
| <i>Spyridia filamentosa</i>                 | W                      | I-2                    | I-2                      | 0                      | 0                       | S-8                            |
| <i>Wrangelia argus</i>                      | I-10, PF               | I-5                    | PF-3                     | I-6, SF                | I-10                    | I-8                            |
| Chlorophyta (green algae)                   |                        |                        |                          |                        |                         |                                |
| <i>Caulerpa racemosa</i> var.               | W                      | 0                      | 0                        | I-6, SF                | I-6                     | I-8                            |
| <i>C. racemosa</i> var. <i>occidentalis</i> | 0                      | 0                      | 0                        | 0                      | 0                       | S-3                            |
| <i>C. taxifolia</i>                         | 0                      | 0                      | SF                       | S                      | I-2                     | I-1                            |
| <i>Codium isthmocladum</i>                  | 0                      | 0                      | SF                       | SF                     | 0                       | 0                              |
| <i>Halimeda opuntia</i>                     | PF-3                   | 0                      | I-4                      | I-2                    | 0                       | I-3                            |
| <i>Udotea flabellum</i>                     | I-2                    | 0                      | I-1                      | 0                      | 0                       | I-2                            |
| <i>Ulva lactuca</i>                         | 0                      | 0                      | 0                        | SF                     | 0                       | S                              |
| Phaeophyta (brown algae)                    |                        |                        |                          |                        |                         |                                |
| <i>Dictyosphaeria favulosa</i>              | 0                      | I-2                    | 0                        | I-2, S                 | I-5                     | I-1, S                         |
| <i>Dictyota Bartayresii</i>                 | 0                      | 0                      | 0                        | I-1                    | 0                       | I-2, S-10                      |
| <i>D. divaricata</i>                        | I-2                    | I-1                    | I-4                      | I-3                    | 0                       | I-1                            |
| <i>D. indica</i>                            | W, PF                  | I-2                    | PF-2                     | I-2                    | I-3                     | I-4, S                         |
| <i>Padina gymnospora</i>                    | I-1                    | 0                      | I-2, SF                  | I-1                    | I-1                     | I-2                            |
| <i>Turbinaria turbinata</i>                 | 0                      | 0                      | 0                        | I-1                    | 0                       | S                              |
| Cyanophyta (blue-green algae)               |                        |                        |                          |                        |                         |                                |
| <i>Lyngbya majuscula</i>                    | W, PF                  | S                      | RF, SF                   | I-6                    | I-2                     | I-10                           |
| Pyrrophyta (dinoflagellates)                |                        |                        |                          |                        |                         |                                |
| <i>Zooanthus</i> sp.                        | W, PF                  | 0                      | I-2                      | SF                     | I-4                     | I-3, S                         |

\* Abbreviations used: I (inhibited growth); S (stimulated growth); SF (stimulated fruiting, i.e. sporulation); RF (reduced amount of fruiting); PF (prevented normal fruiting); W (hyphae and sporangia very white); 0 (no effect on growth); numerals (width in mm of affected zone around filter paper disc). Double or triple entries indicate a multiple response, either on the same plate or on different plates.

to the otherwise dark colored *Rhizopus* plates. The hyphae generally grew up to the edge or very near the edge of the filter-paper disc, and spores were formed as usual. The only change appearing grossly was the complete loss of pigmentation and the stark whiteness of the hyphae and spores. The significance of these observations is not as yet understood.

#### DISCUSSION

This preliminary survey of the most common marine algae in the vicinity of La Parguera, Puerto Rico indicates that these organisms in

some instances contain substances having anti-fungal activity. Of the 35 species tested, almost all showed at least a trace of activity against one or more of the test organisms. *Laurencia obtusa*, *Wrangelia argus*, and *Lyngbya majuscula*, two red algae and a blue-green alga, gave the most consistently positive and striking results against the molds. *W. argus*, *L. majuscula*, and *Laurencia papillosa* were very effective against the yeasts, yielding zones of inhibition of 10 to 12 mm wide. One sample of *Caulerpa racemosa* var. was very active against *Candida* and *Cryptococcus*, although others were entirely without activity.

*Zooanthus* sp., a dinoflagellate-containing anemone, was active against *Candida* and gave very wide white zones on the otherwise dark *Rhizopus* plates. Other species displaying less activity were *Amphiroa fragilissima*, *Dictyosphaeria favulosa*, *Dictyota divaricata*, *D. indica*, *Halimeda opuntia*, and *Hormothamnion enteromorphoides*. Species which showed no significant inhibition or stimulation of the test fungi were as follows: *Acanthophora specifera*, *Caulerpa cupressoides*, *C. racemosa* v. *microphysa*, *C. setularioides*, *Centroceras clavulatum*, *Chondria littoralis*, *Cladophoropsis membranacea*, *Halimeda monile*, *Penicillus capitatus*, *Sargassum platycarpum*, and *Valonia ventricosa*.

Inhibitory activity does not seem to be limited to any particular group of algae, nor to any particular habitat. Members from five divisions of marine algae (reds, browns, greens, blue-greens, and dinoflagellates) were shown to inhibit selected species of pathogenic fungi, and it is believed that further research will turn up other inhibitors. \*

Although preliminary, these results are very encouraging. The need for systematic sampling of marine organisms, in a general search for antibacterial and fungicidal substances, is strongly indicated by both the literature and the results of this study.

In Puerto Rico, further sampling should be done among the marine algae and the algae-containing anemones and corals. An attempt should also be made now to isolate and, if possible, identify, or at least characterize, the inhibitory substances from the most potent inhibitors: *Laurencia obtusa*, *L. papillosa*, *Lyngbya majuscula*, and *Wrangelia argus*. The red tide organism, *Goniaulax tamarensis*, which Burkholder et al. (1960) found to inhibit *Candida*, should also be studied further. Unfortunately, it was not available during this trip.

#### ACKNOWLEDGMENTS

The author is indebted to Luis R. Almodovar for aid in the collection and identification of the algae and to Juan Rivero, Director of the Institute of Marine Biology, University of Puerto Rico, Mayaguez, for making the facilities of the Institute's La Parguera field station available.

Aided by grants ONR Nonr-3203 (00) and E-861, National Microbiological Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Maryland.

#### LITERATURE CITED

- ALLEN, M. B., AND E. Y. DAWSON. 1960. Production of antibacterial substances by benthic tropical marine algae. *J. Bacteriol.* **79**:459-460.
- BURKHOLDER, P. R., L. M. BURKHOLDER, AND L. R. ALMODOVAR. 1960. Antibiotic activity of some marine algae of Puerto Rico. *Botan. Marina* **2**:149-156.
- CHESTERS, C. G. C., AND J. A. STOTT. 1956. The production of antibiotic substances by seaweeds. Second International Seaweed Symposium. Pergamon Press, New York.
- MAUTNER, H., G. M. GARDNER, AND R. PRATT. 1953. Antibiotic activity of seaweed extracts. II. *Rhodomela larix*. *J. Am. Pharm. Assoc., Sci. Ed.* **42**:294-296.
- PRATT, R., T. C. DANIELS, J. J. EILER, J. B. GUNNISON, W. D. KUMLER, J. F. ONETO, L. A. STRAIT, H. A. SPOEHR, G. J. HARDIN, H. W. MILNER, J. H. C. SMITH, AND H. H. STRAIN. 1944. Chlorellin, an antibacterial substance from *Chlorella*. *Science* **99**:351-352.
- PRATT, R., H. MAUTNER, G. M. GARDNER, HSIEN SHA, AND J. DUFRENOY. 1951. Antibiotic activity of seaweed extracts. *J. Am. Pharm. Assoc., Sci. Ed.* **40**:575-579.
- VACCA, D. D., AND R. A. WALSH. 1954. The antibacterial activity of an extract obtained from *Ascophyllum nodosum*. *J. Am. Pharm. Assoc., Sci. Ed.* **43**:24-26.