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Antifouling Agents From Eelgrass: Identification, Characterization and Mode of Action

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13. ABSTRACT *(Maximum 200 words)*

This research has focused on the isolation and structural characterization of natural, non-toxic antifoulants produced by the eelgrass Zostera marina L. Chemical purification of a single active agent from fractions produced by aqueous-organic phase partitioning, size filtration chromatography and HPLC was directed by microbial attachment assays. Structural characterization of the isolated agent was accomplished by high resolution nuclear magnetic resonance and mass spectrometry. The isolated compound and several analogs were synthesized. Laboratory bioassays and short term (<10 day) field experiments indicate that the natural agent and at least of the one synthetic analogs show significant antifouling activity against marine bacteria, algal spores, benthic diatoms, tube worms and barnacles. Evaluation of their antifouling potential continues. A patent application describing the use of these compounds as non-toxic antifouling agents is in preparation.

14. SUBJECT TERMS
Biofouling, Natural Products, Eelgrass, Antifouling

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GOALS:

The overall goals of this research have been to identify naturally-occurring, non-toxic antifoulant roduced by the marine angiosperm, *Zostera marina* L. (eelgrass) and to characterize the mode of action of these agents in preventing attachment of fouling organisms.

NEAR TERM OBJECTIVES:

Our objectives have focused on the isolation and structural characterization of the active agent(s), chemical synthesis of the isolated compound and several structural analogs, and evaluation of the antifouling effectiveness of the compounds. We have also screened extracts from other macrophyte taxa for the presence of antifouling activity and collected preliminary data on the antifouling effectiveness of the agent against hard foulers in the field.

APPROACH:

Purification of a single active agent, using a sequence of aqueous-organic phase partitioning, size filtration chromatography and high pressure liquid chromatography (HPLC), was directed by a bacterial attachment bioassay. Structural characterization of the agent was accomplished by high resolution nuclear magnetic resonance and mass spectrometry.

TASKS COMPLETED:

The active agent isolated from eelgrass has been purified and characterized structurally. This compound, along with several analogs, have been synthesized. Evaluation of the antifouling potential of these compounds continues. A patent application is in preparation for the use of the natural compound and synthetic analogs as non-polluting antifouling agents.

RESULTS:

The synthetic compounds showed similar activity to the agent purified from eelgrass. Bacterial attachment to slides treated with one of the synthetic agents decreased exponentially with concentration (Fig. 1). The IC_{50} (concentration causing a 50% inhibition in bacterial density) was about 1 $\mu g\ ml^{-1}$ (12 $\mu g\ cm^{-2}$ of treated surface). Despite the strong antifouling effect, there was no evidence of toxicity to bacterial growth in liquid culture or on agar at concentrations up to 10x higher than the IC_{50} dose. Thus, the therapeutic index (IC_{50}/LD_{50}) remains undefined but extremely high (≥ 100).

Soluble extracts were prepared from other local macrophytes and screened for antifouling activity. Of the 8 spp examined, including representative taxa from all major macrophyte groups, only extracts of *Zostera marina* showed significant activity (Fig. 2). Thus, the activity exhibited by the eelgrass extract was not a widespread phenomenon.

Preliminary field data indicate that the crude eelgrass extract effectively inhibited attachment of spirorbid worms (polychaetes) and colonial ascidians to unglazed ceramic tiles, while it was not effective against solitary tunicates (Fig. 3). No barnacles settled on any tiles during the course of this experiment.

The crude extract and a synthetic analog were also effective against barnacle larvae in laboratory attachment assays. The IC_{50} against barnacle attachment was about 1 $\mu g/ml$ (12 $\mu g/cm^2$ of treated sfc.), similar to that demonstrated for bacteria (Fig. 4). As with bacteria, these agents were not toxic to barnacle larvae at concentrations that inhibited attachment.

ACCOMPLISHMENTS:

The chemical agent isolated from eelgrass leaves has been purified and characterized structurally. It is effective at preventing attachment of a wide range of fouling organisms, from bacteria to barnacles, at concentrations that are orders of magnitude below the lethal dose. The agent is structurally simple, and has proven easy to synthesize from readily available and inexpensive substrates. Several manuscripts and a patent application are in preparation from this work.

PUBLICATIONS SUPPORTED BY THIS RESEARCH:

Kopczak, C.D., Zimmerman, R.C. and Kremer, J.N. 1991. Variation in Nitrogen Physiology and Growth Among Geographically Isolated Populations of the Giant Kelp, Macrocystis pyrifera (Phaeophyta). J. Phycol. 27:148-158.

Zimmerman, R.C. and Alberte, R.S. 1992. Regulation of Carbon Partitioning in the Seagrass. Zostera marina L. (eelgrass). I. Assimilate Export Rates, Enzyme Activities and the Effect of Root Anoxia on Carbon Transport. Plant Physiol. In review.

Todd, J.T., Zimmerman, R.C. Dea, C.H., Crews, P., and Alberte, R.S. 1992. Isolation of natural antifouling agents from Zostera marina L. (eelgrass) and the antifouling activity of synthetic analogs. Phytochemistry. *In prep.*

Zimmerman, R.C. Todd, J., Dea, C.H. Baier, R., Crews, P., and Alberte, R.S. 1992. Mechanisms of Fouling Avoidance in Zostera marina L. (eelgrass). Mar. Biol. In prep.

PATENTS RESULTING FROM THIS RESEARCH:

Zimmerman, R.C. and Alberte, R.S. In Prep. Antifouling properties of natural zosteric acid isolated from eelgrass, and synthetic analogs. Arch Development Corp., Univ. Chicago, Chicago, IL 60637.

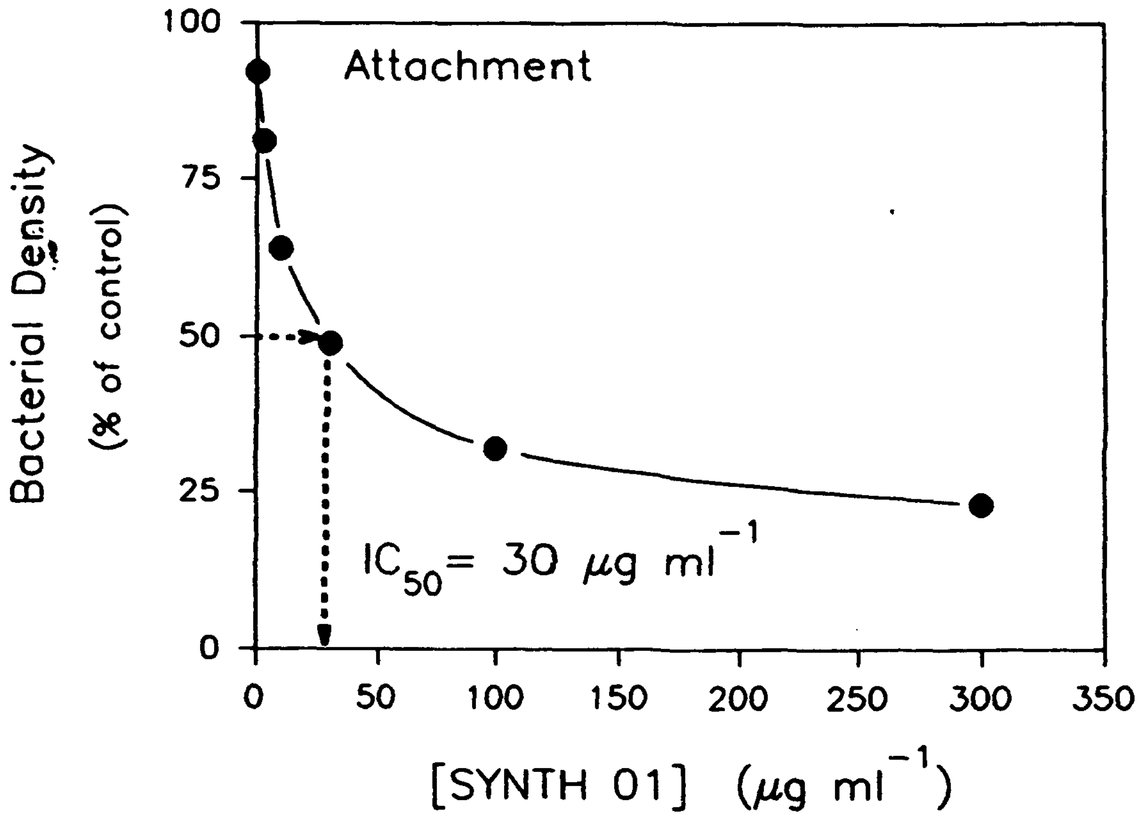

Figure 1. Effect of concentration of a synthetic antifouling agent on attachment density of bacteria in laboratory assay. The IC_{50} concentration is about 1 μg ml^{-1} (12 μg cm^{-2} of treated surface).

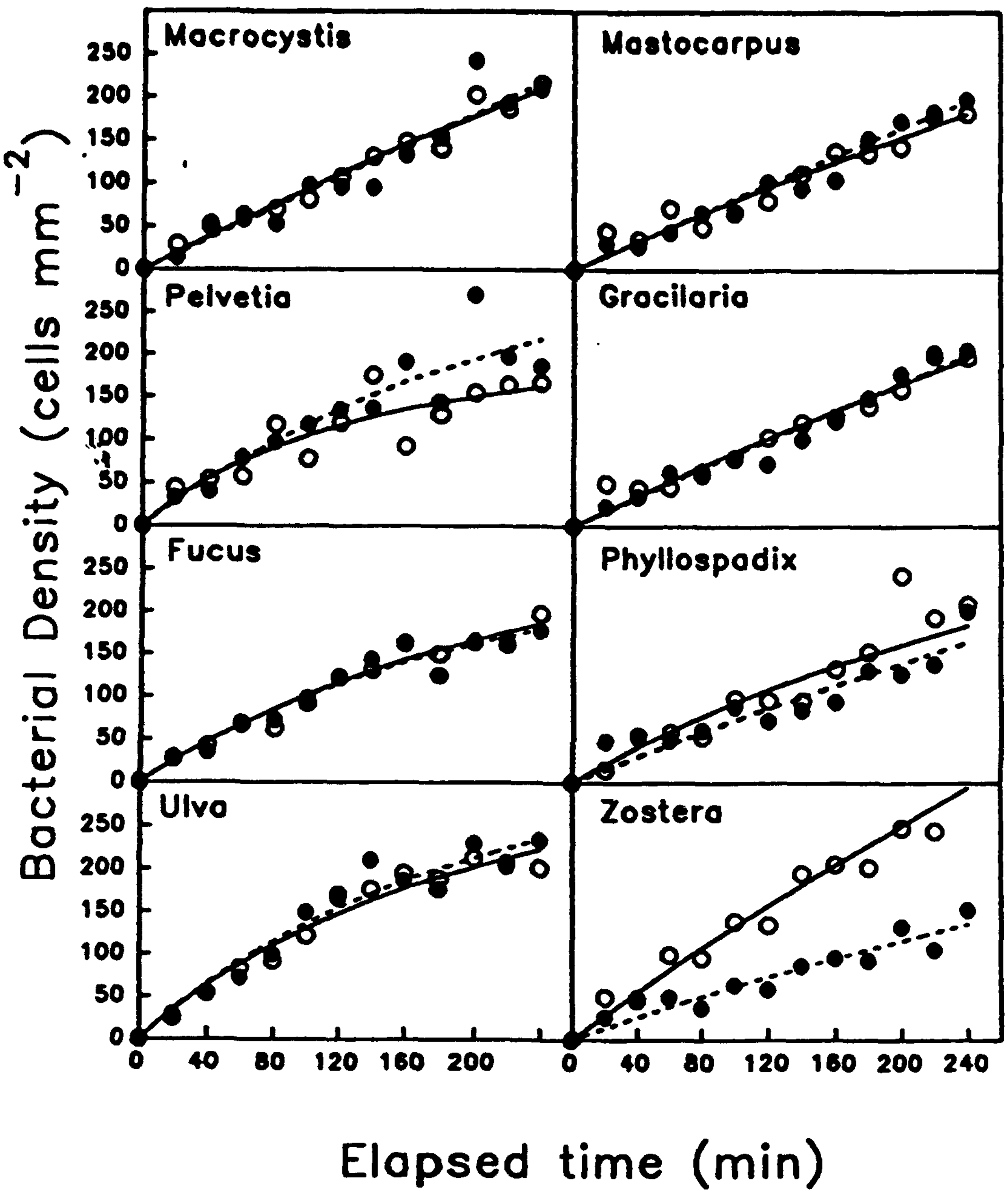

Figure 2. Results from bacterial attachment assays with extracts prepared for different marine macrophytes. Slides treated with solvent controls are indicated by open circles (O), slides treated with extract are indicated by filled circles (●).

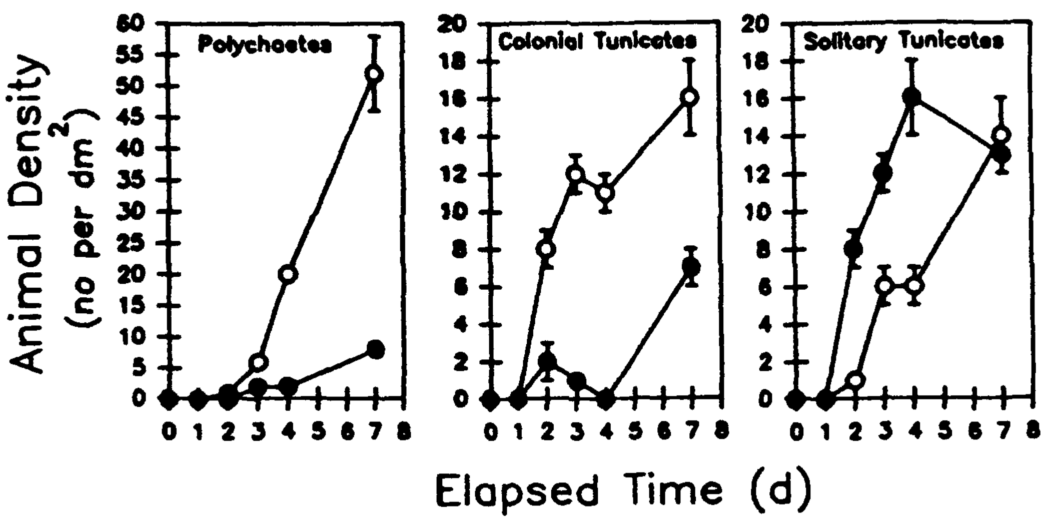

Figure 3. Effect of the crude eelgrass extract on settlement density of 3 invertebrate taxa in the field. Solvent control tiles are indicated by the open circles (O), extract-treated tiles are indicated by filled circles (●).

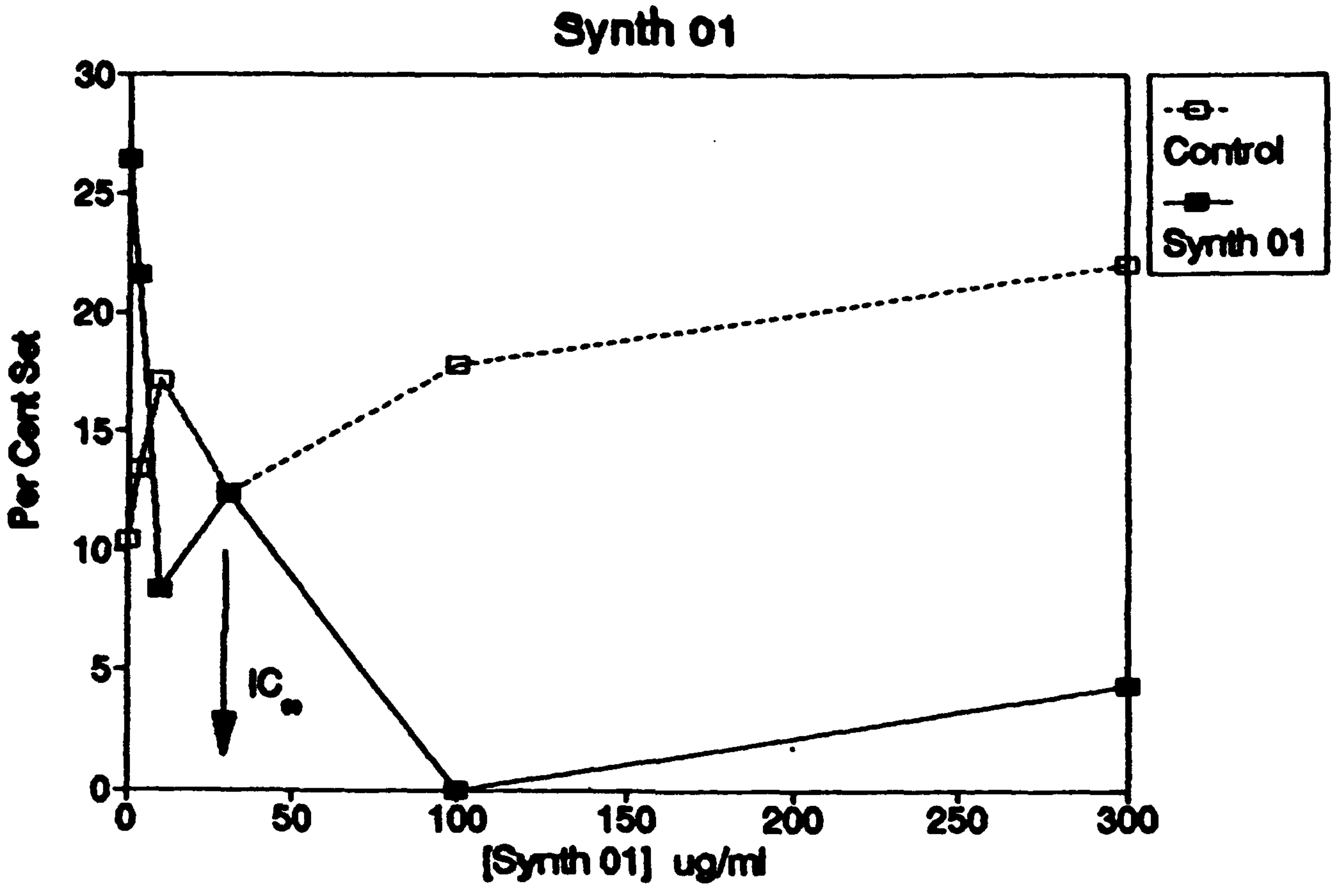

Figure 4. Effect of concentration of a synthetic antifouling agent on barnacle settlement in the laboratory. The IC_{50} for barnacles was about 1 $\mu g\ ml^{-1}$ (12 $\mu g\ cm^{-2}$ of treated surface), similar to that for marine bacteria.

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