# CHEMICAL DEFENCE AND ANTIFOULING ACTIVITY OF SPONGES OF THE GENUS IRCINIA

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## Abstract

The defense roles and the antifouling activity of the organic extracts and the major metabolites of the sponges *Ircinia pausifilamentosa*, *I. variabilis* and *I. spinosula* were investigated. The antifeedant activity was tested on the generalist predator fish *Thalassoma pavo*. The antifouling activity was tested against representatives of the three major groups of fouling organisms, marine bacteria and marine fungi, diatoms and the blue mussel. All extracts showed promising levels of activity.

Key Words : Porifera, Aegean Sea, Predation,

#### Introduction

Sponges are important members of the marine ecosystem. Because they are sessile and soft-bodied, they appear to be physically vulnerable. The lack of predation on sponges is thought to result from the elaboration of physical and chemical defenses.

A number of studies have shown that organic compounds extracted from a diversity of non-motile marine taxa, including sponges inhibit fouling in the laboratory or in the field. It has been stated (1) that these organisms secrete chemicals that prevent larvae of other marine organisms from settling and growing on them. The sponges of the genus *Ircinia* found in shallow Mediterranean marine ecosystems are most of the times free of epibionts and have at the same time proven to be rich sources of interesting metabolites.

# Materials and Methods

Sponges were collected by SCUBA diving from the island of Melos and the island of Fleves. The preliminary palatability of the sponges was evaluated in laboratory aquaria with the generalist predator fish *Thalassoma pavo. Ircinia* extracts and pure metabolites were incorporated in food preparations following the methods described by Pawlik







2-(24- hydroxy)Octaprenylo hydroquinone 2-Octaprenylo 1,4-1,4hydroquinone



Ircinia variabilis metabolites

# Ircinia pausifilamentosa metabolites



Major metabolites of the investigated Ircinia species.

## (2).

Antibacterial testing of the extracts was performed by the disc diffusion technique in agar plated petri dishes (3). Five Gram-positive bacteria and five Gram-negative bacteria were chosen for this study. The activities of the sponges extracts and compounds towards marine fungi strains were performed using a modified well-agar diffusion method. Five marine fungi strains were obtained from the Culture Collection of the University of Portsmouth.

Inhibition of microalgae growth was tested on Diatomophyceae strains obtained directly from the Culture Collection of Algae of the University of Caen, including *Amphora coffeaformis*, *Phaeodactylum tricornutum*, *Cylindrotheca closterium*. The screening of activity was performed as previously described in Sawant & Garg (4). Antifouling tests were performed against three macroalgae namely *Enteromorpha intestinalis*, *Ulva lactuca* and *Sargassum muticum*. Samples were collected in Concarneau Bay, France. Spores liberated from fronds after an incubation at 25°C during 20 min. were collected in a beaker and used for the experiments (4). The antifouling activities of the extract toward Mytilus edulis was measured by recording the activity of the phenoloxidase as previously described in Hellio *et al.* (4). Toxicity tests on oyster (*Crassostrea gigas*) and sea urchin larva (*Echinus esculentus*) were realised as previously described (4). The biocide TBTO (10 ppm) was used in all assays as a standard to check the sensitivity (4)

### **Results and Discussion**

The defense mechanism of the sponges was found to rely on the presence of the major metabolites such as Ircinin I and II for *I. pausi-filamentosa* and variabilin for *I. variabilis*. The extract of *I. spinosula* showed only moderate activity and that was traced to the prenylated hydroquinone constituents. The active factors of *I. pausifilamentosa* and *I. variabilis* were tested in natural concentrations and were inhibiting higher than 80% of the fish feeding.

Most of the extracts exhibited significant activity against fouling, with strong effect on the three major groups of fouling organisms (micro-organisms, algae and invertebrates). The dichloromethane extract of *I. spinolusa* and ethanol extract of *I. pausifilamentosa* were relatively broad spectrum, showing high to moderate level of activity in all assays. Since every antifouling coating must work against a range of fouling organisms, the above activities are promising.

#### References

1. Fusetani, N. 1991. The Fusetani biofouling project. *Biofouling*. 12: 2-8.

Pawlik, J. R., Chanas, B., Toonen, R. J., and Fenical, W. 1995.
Defences of Caribbean sponges against predatory reef fish: I. chemical deterrency. *Mar. Ecol. Prog. Ser.* 127: 183-194.
Devi, P., Solimabi, W., D'Souza, L., Sonak, S., Kamat, S., and Singbal,

3. Devi, P., Solimabi, W., D'Souza, L., Sonak, S., Kamat, S., and Singbal, S. 1997. Screening of some marine plant for activity against marine fouling bacteria. *Bot. Mar.* 40 : 87-91.

4. Hellio, C., Bremer, G., Pons, A.M., Le Gal, Y., and Bourgougnon, N. 2000. Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany (France). *Appl. Microb. Biotech.* 54 (4) : 543-549.

Rapp. Comm. int. Mer Médit., 36, 2001