BIOSYNTHETIC ORIGIN OF FURANOSESTERTERPENES IN THE SPONGE IRCINIA FELIX.

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Abstract

The present results strongly suggest that heterotrophic bacteria found in great abundance within the sponge tissues are involved in the production of furanosesterterpenes in *Ircinia felix*. Taken together, their increase after inflicted injuries and their antibacterial activity indicate that they might play a role in limiting bacterial proliferation during the process of wound healing.

Keywords: Bacteria – antibiotics – secondary production – Porifera

Sponges are a source of a great diversity of secondary metabolites presumably used for antipredation, competition for space and control of invading microorganisms. Some of these natural products may be of use for man as drugs, anti-fouling substances and a variety of other functions. Many sponges harbour large numbers of prokaryotic endobionts and it is often assumed that they may be involved in the production of these substances. In this context, we have investigated the marine sponge *Ircinia felix*. This species, common in the Caribbean Sea, is known to contain numerous bacteria and cyanobacteria and to produce bioactive compounds, in particular furanosesterterpene tetronic acids (1). The aim of this study was to identify the biosynthetic origin of these metabolites and to understand their ecological significance, especially after wounding.

Sponges were collected by SCUBA diving in the Caribbean Sea off Curaçao at 30 m. They were immediately transported in seawater to the laboratory where they were processed. A piece of the ectosome and the choanosome were carefully cut off and mechanically dissociated through a 100 μm nylon mesh into cold Ca^{++} / Mg^{++} - free artificial seawater, 10 mM EDTA. The remaining part was put back into the sea for 24 h, and then the sponge tissues were again dissociated as described above. The endosomal and ectosomal cell suspensions from the intact and injured specimens were centrifuged for 10 mins, respectively at 300 g (C1 fraction) and 1000 g (C3 fraction); the corresponding supernatants were further centrifuged at 4000 g for 15 mins (C2 and C4 fractions). Phase-contrast and UV microscopy observations showed these cell fractions to be enriched either in sponge cells (C1), cyanobacteria (C3) or bacteria (C2 and C4). However bacteria present in large amounts in sponge tissues were still present in significant numbers in the C1 and C3 fractions. On the contrary, cyanobacteria were almost absent from C1 and C2 choanosomal fractions.

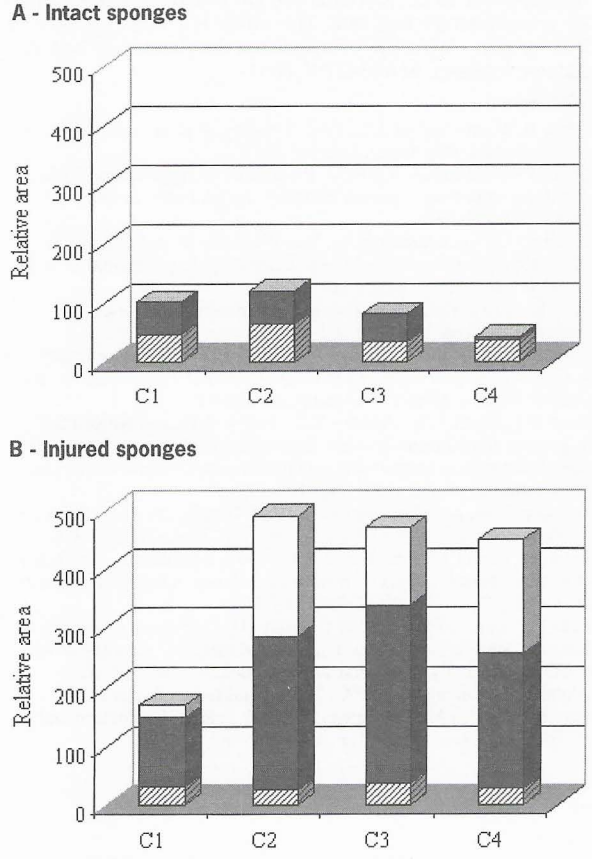

Fig. 1. Distribution of metabolites in cell fractions (relative area calculated to MeOH as internal standard).

The extraction of the cell fractions with methanol followed by Reverse Phase HPLC analyses revealed the existence of two main groups of secondary metabolites, furanosesterterpenic derivatives of the variabilin-type (V1 and V2) and sulfircin-like compounds (S). No significant differences of S content were observed between fractions before and after wounding (A, B). On the contrary, 24 hours after wounding, a significant rise of V1 was observed as well as high amounts of V2 detected only in the injured sample (B). (V1 + V2) /S ratios increased, several-fold principally in the C2, C3 and C4 fractions.

These findings agree with those of Zea (3) who observed a strong increase of variabilin in *Ircinia felix* tissues when sponges were purposely injured. Our results extend these observations at the cellular level and suggest that heterotrophic bacteria rather than sponge cells or cyanobacteria would be responsible for the production of variabilin-type furanosesterterpenes. The fact that these metabolites occur in different species and genera of the family Ircinidae and the Orders Dictyoceratida/ Dendroceratida together with the presence of a constant large number of bacteria in *I. felix* give further support to our proposal.

Standard disc bioassays (500μg/disc) showed a moderate antibacterial activity of C2, C3, C4 and to a lesser extent C1 fractions against *Escherichia coli* but no activity against *Bacillus subtilis* and *Saccharomyces cerevisiae*. The antibacterial activity appeared slightly enhanced in injured sponges. These results indicate that furanosesterterpenes may act as internal antibiotic protection as suggested by Zea (3).

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References

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