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Advances in the production of sponge biomass *Aplysina aerophoba*—A model sponge for ex situ sponge biomass production

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Abstract

Sponges are a promising source of organic compounds of potential interest regarding industrial and medical applications. For detailed studies on such compounds, large amounts of sponge biomass are required. Obtaining that is at present extremely difficult because most sponges are relatively rare in nature and their mass cultivation in the laboratory has not yet been accomplished. In this study the possibility of culturing *Aplysina aerophoba* fragments in laboratory was examined. While a substantial biomass increase was not yet observed, we achieved fragmented sponge tissue to develop into a functional sponge as a first success.

Keywords: Sponge cultivation; *Aplysina aerophoba*; Marine natural products supply

1. Introduction

1.1. Sponges

Sponges (Porifera) are simple animals characterized as sessile active filter feeders. They represent the oldest metazoan phylum. The entire sponge tissue is specialized for filtering and exhibits no distinct organs. Sponges feed by extracting suspended or dissolved food from the surrounding water, using flagellated cells, the choanocytes. The structure of these

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choanocytes, which constitute the basic pumping and filtering elements, is the same in all sponges (Larsen and Riisgard, 1994). Virtually all sponges harbor an associated microbial community. The amount of microorganisms, however, can vary considerably. Various microorganisms have been found associated with sponges, including cyanobacteria, diverse heterotropic bacteria, archaea, unicellular algae and zoochlorellae.

1.2. Why sponge biomass production?

Among marine invertebrates, sponges have yielded the largest number of interesting natural products so far. Several sponge compounds proved to be of interest for drug development (Kobayashi, 2000).

However, the development of drugs from marine natural products in general, and particularly from sponge metabolites, is frequently prevented by severe supply limitations. The majority of promising compounds have complex structures, thus limiting their availability by chemical synthesis. Further, the yields from the respective organisms are generally minuscule. Therefore, for detailed studies on such compounds, large amounts of sponge biomass are required. Obtaining that is at present extremely difficult because most sponges are relatively rare in nature and their mass cultivation has not yet been accomplished. This results in a major problem for extended tests in the courses of pharmaceutical developments, which require bulk amounts of sponge compounds. It is generally accepted that extensive harvesting of sponges in the wild is not a feasible solution of this problem. This problem is often ignored by marine natural product research, as the emphasis mostly lies on the discovery of new bioactive natural products (Fusetani, 2000; Faulkner, 2000).

From the point of view of biotechnological production, three groups of sponge-derived compounds can be distinguished:

- Direct gene products, proteins, which can usually be produced by recombinant expression in appropriate microorganisms.
- Secondary metabolites of sponge-associated microorganisms which can be obtained by pure culture of the respective microorganisms after isolation from the sponge.
- Sponge-specific secondary metabolites which are only available directly from sponge biomass.

Regarding the availability of the latter metabolites, it is only of secondary interest whether the respective metabolites originate from the sponge cells or from uncultivable sponge-associated microorganisms, or whether they are a result of a metabolic interaction of the sponge and the associated microorganisms. In view of the great potential of sponge-derived compounds, there has been only very limited work on the cultivation of functional sponges, sponge tissue or sponge cells. Today, biotechnological sponge cultures are still a challenging task, as it is difficult to cultivate sponges under adequate but well defined growth conditions. As a result, sponge-specific metabolites are still not available by biotechnological means (Belarbi et al., 2003; Osinga et al., 1999a,b,c, 1998; Kinne, 1977). However, a completely controlled biotechnological cultivation of sponges promises substantially higher growth rates, as compared to those in natural environments or aquaculture. To achieve this goal is a first step towards a biotechnological production of sponge-specific metabolites.

1.3. Strategies for the production of sponge biomass

The aim of the study presented here is to develop a model system for the biotechnological cultivation of functional sponges. The aspects which are assumed to be important to sponge growth regarding any species are water and food quality and quantity, as well as the composition of the associated microorganisms contained in the cultured sponges.

Prior to the development of a special sponge cultivation process it must be realized that it is unknown which culture parameters are crucial. It is notoriously difficult to keep these animals in a laboratory, and most sponges are prone to display a loss of biomass and ultimately die. This implies that for any kind of research, a supply of individuals has to be assured, either by collecting from nature or by harvesting aquacultures.

While it is difficult to sustain sponges under controlled, defined conditions in laboratory experiments, sponges often can be found in running unfiltered sea water or in large public aquaria or related large volume sea water systems. However, it can be assumed that such species, apparently growing under artificial conditions, were unintentionally selected. As many sponge species are introduced into these systems, probably