

In vitro reagggregates of mediterranean sponges (Porifera):

Preliminary studies of biomass production for subsequent extractions of bioactive substances

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Abstract

Sponges (*Porifera*) are not only interesting regarding their morphology, their way of living or their cytology. Since we have knowledge about their production of chemical antibodies against predators and microbial settlement, different endeavours have been made to extract the bioactive substances. These compounds represent potential pharmaceuticals like cytostatics, antibiotics or anti-viral and anti-inflammatory agents.

Besides chemical synthesis and aquacultures of sponges, biotechnological methods like cell and tissue cultures are of high interest. They are cost-efficient, reproducible and practicable in laboratories far away from the ocean. Principal problems are the production of capable medium and sufficient food supply for the cultures.

Since cell-cell contacts are needed for the intercellular communication of the sponge cells, which amongst other things is necessary for the proliferation activity, a new approach for tissue culture was tested in the present study.

We tested to which extent dissociated solitary cells are able to reaggregate and if they are applicable for the cultivation of multicellular aggregates (MCAs) in the laboratory. For these purposes eight different sponges of the mediterranean littoral were observed.

The methods used for the dissociation play an important role. Two different methods were applied. For each species a mechanical and a chemical dissociation technique was carried out.

Variations between individuals of different sponge species treated with one method, as well as differences between individuals of the same species treated with the two methods already emerged regarding the dissociation yield and reaggregation behaviour. Best results were achieved with the mechanical method: Reagggregates of *A.verrucosa* and *P.ficiformis* were round and showed a smooth surface. The chemical method led to frazzled, irregular aggregates, which additionally showed increased microbial contamination and ultimately perished.

The aggregates of *P.ficiformis* showed a specialty. They possessed a plasticity allowing them to spread and flatten on the bottoms of Petri dishes and on cover slips. During the observation of this plasticity, locomotion of different cell types

of the MCAs were noticed which hitherto were only known for living, intact sponges. We tried to identify the cell types building up such MCAs and tried to give future prospects for the biotechnological production of sponge biomass. Thus, handling of MCAs of *P.ficiformis* was tested in a perfusion-chamber with man-made seawater functioning as medium. In this experiment proliferation of sponge cells could not be detected; after 2-3 weeks, diatoms, introduced by mistake, dominated the culture.

Nevertheless, when using appropriate medium with special food composition, such a chamber could be a promising system for the cultivation and production of sponge tissue. The great advantage is the evacuation of waste products (given into the medium by the sponge cells), avoiding possible inhibition of growth.