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Journal of Experimental Marine Biology and Ecology 337 (2006) 171–177

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Seasonal production of primmorphs from the marine sponge *Petrosia ficiformis* (Poiret, 1789) and new culturing approaches

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Received 6 March 2006; received in revised form 20 May 2006; accepted 19 June 2006

Abstract

The need to produce bioactive compounds from marine sponges leads several groups of research to the culture of primmorphs from different species, which are generally maintained in aquaria for long time before processing. Here we present a study where the importance of several parameters on primmorphs production from the symbiotic sponge *Petrosia ficiformis* has been evaluated: (i) the sterility of sea water, (ii) the maintenance in aquarium before processing, (iii) the seasonal cycle. Sterility of sea water does not improve primmorphs production in this species. The maintenance of sponges in aquaria before processing negatively affects cell cultures. Regarding seasonality, it is evident that both the number and the size of primmorphs can deeply change depending on the period of the year the sponge is collected. April and July are the months that lead to the highest number of primmorphs, May and June are the months that lead to their biggest sizes. Possible relationships of these results with the life cycle of *P. ficiformis* are discussed.

Keywords: Cell culture; Mediterranean sponge; Primmorphs; Seasonal cycle

1. Introduction

Primmorphs, round shaped three-dimensional aggregates of cells, can be obtained from sponges after cell dissociation with mechanical or chemical methods and represent one of the most promising approaches to product sponge biomass for biotechnological applications (Muller et al., 1999; Custodio et al., 1998). This is due to the occurrence of totipotent cells able to maintain telomerase activity (Koziol et al., 1998; Muller et al., 1999). However sponge cell culture exhibits a variety of difficulties, for example there are no sponge portions, with the exception of larvae (Leys et al., 2005), from which sterile primary cultures can be obtained. Therefore without sterility or addition of antibiotics, cultures are contaminated by proliferating bacteria and protozoa within 1–3 days (Pomponi and Willoughby, 1994). On the other hand, it seems that antibiotics negatively affect primmorphs formation (Sipkema et al., 2003).

Up to now, among sponge species tested to produce primmorphs, *Suberites domuncola* (Le Pennec et al., 2003; Krasko et al., 2000; Sipkema et al., 2003; De Rosa et al., 2003), *Dysidea avara*, *Stylotella agminata* (Zhang et al., 2003) and *Petrosia ficiformis* (Pozzolini et al.,

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doi:10.1016/j.jembe.2006.06.030

2004) are those better investigated. In a recent paper Valisano et al. (2006) compared the ability to produce primmorphs among several Mediterranean sponge species and *P. ficiformis* resulted one of the most productive.

Due to the aptitude of *P. ficiformis* to produce primmorphs and to its ability to synthesize polyacetylenic compounds, with diverse biological activities such as antimicrobial, antifouling, H^+, K^+-ATPase inhibitory, HIV inhibitory, immunodepressive and antitumor activities (Fusetani et al., 1993; Hallock et al., 1995), we performed several experiments to find the optimal approach to culture primmorphs from this species.

We focus our attention on (i) the effect of sterile/non sterile sea water on primmorphs formation dynamics; (ii) the effect of maintenance in aquarium before processing; (iii) the importance of the seasonal cycle of the species on primmorphs formation.

2. Materials and methods

2.1. Specimens collection

Specimens of *Petrosia ficiformis* were collected along the Marine Protected Area of Portofino (Ligurian Sea, Italy) between 15 and 20 m depth. Sponges were immediately carried to the laboratory and maintained in aquaria at 12 °C and salinity at 38‰.

2.2. Cell dissociation

The day after sampling, sponges were processed for the dissociation of cells according to the protocol established by Müller et al. (1999). Sponge samples of 4 to 5 cm^3, continuously submersed in sea water, were cut in small pieces and transferred into 50 ml conical tubes filled with CMFSW–EDTA. After gentle shaking for 20 min, the solution was discarded and new CMSFW–EDTA was added. After continuous shaking for 40 min the supernatant was collected and filtered through a 40 μm mesh nylon net and the process repeated once. Samples were centrifuged (1600 rpm, 458 *g* for 5 min) and washed twice in CMFSW. Cells in final pellets were re-suspended in natural filtered sea water and dissociated cells were put into tissue culture plastic plates into six replicates on an oscillating table to avoid cells to attach on the bottom of plates. Every three days one third of seawater was replaced with new filtered one and for three weeks the formation of primmorphs was monitored, considering the number and the size of primmorphs that were measured under a stereomicroscope. According to Sipkema et al. (2003) areas of primmorphs were calculated assuming that primmorphs are perfect spheres.

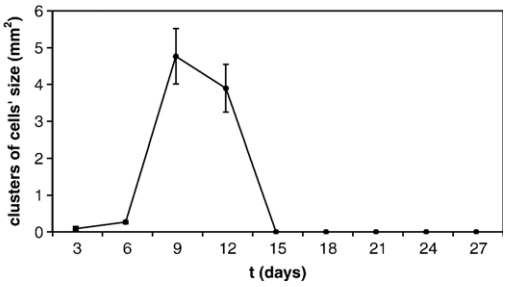

Fig. 2. Cultivation of cells from *Petrosia ficiformis* after 15 days of maintainance in aquaria: no primmorphs are obtained but only clusters of cells.

2.3. Experimental and statistical analyses

At the start of the experiments (January 2004) to test the effect of sterility on cell cultures, we compared cultures of cells performed in two experimental sets, one using filtered and sterile seawater and the other one using filtered but non sterile one. We did six replicates for each experimental condition.

To test the influence of a period of maintenance in aquarium on the formation of primmorphs, sponge samples

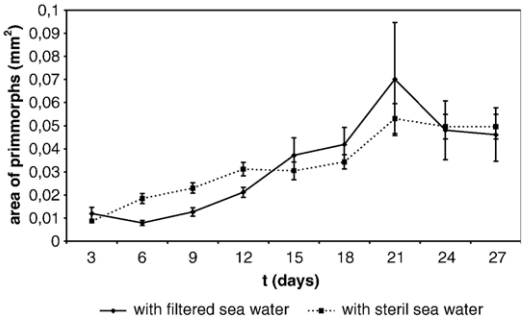

Fig. 1. Comparison between the trends of growth and dimensions reached by primmorphs of *Petrosia ficiformis* cultivated with sterile and filtered sea water.

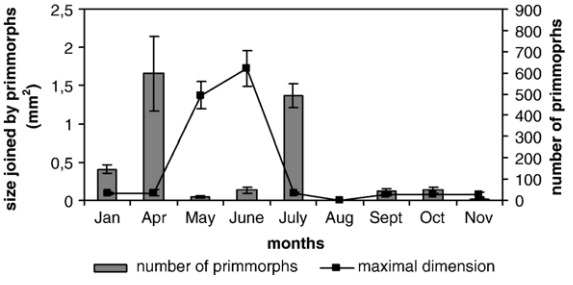

Fig. 3. Seasonal variation of number and size of primmorphs from *Petrosia ficiformis* during an annual cycle.