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Practical and descriptive techniques for *Gelidium rex* (Gelidiales, Rhodophyta) culture

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Abstract

Research on the culture of *Gelidium rex* was approached from two points of view, growth of thalli from spores and growth from re-attachment. Mollusk shells, which are very easy to handle, were used in both systems. The results achieved by these methods showed that reattachment is the technique that obtains larger plants in a shorter time. This is the first stage in developing culture and cultivation techniques aimed at commercial exploitation of this species in Chile.

Introduction

Interest in *Gelidium* spp. is constantly increasing worldwide, due to the high value of its polysaccharide, as well as the demand for its by-products, such as agarose, which has multiple uses in genetic engineering. This interest is currently supported by the relatively fragile natural populations and the strong cyclic fluctuations occurring with the crop of *Gelidium* spp. and the production of agar (Salinas, 1991; Santelices, 1988, 1989).

In Chile, there are three species of commercial importance, *Gelidium rex* Santelices et Abbott, *G. lingulatum* Kuetzing and *G. chilense* (Montagne) Santelices et Montalva. Although these species show continuous reproduction all year round, the biomass volumes measured in each unit of area are generally low. In experiments where the algae are removed and trimmed, these species have shown rather slow growth, especially if the trailing axes of the thalli are destroyed during cropping (Alveal et al., 1990; Santelices, 1989).

Considering this information, which indicates that an increase in biomass of these algae is required, plus their commercial importance, in April 1994 a research project was begun in an attempt to cultivate this resource. This report includes the results of the first steps in this project.

Materials and methods

Culture from spores

Reproductive material of *Gelidium rex* was collected from the field at 'Punta Lengua de Vaca', Bahía Tongoy (30°13′13″ S, 71°34′00″ W), Chile. The material was taken to the laboratory under humid and cool conditions, where a selection of the algae was performed. A shock treatment was applied to induce sporulation: thalli were placed on drying paper at 16 °C for 24 h in darkness.

Once the induction period was over, the algae were placed in a 0.5 m^3 fiberglass tank, suspended 30 cm from the bottom of the tank by 4 mm plastic mesh, so that the limpet shell substratum (*Fisurella* sp.), placed on the bottom of the tank, would receive a rain of spores.

The substratum inoculation lasted 48 hours, after which the algae were removed and the seawater in the tanks was changed.

At this stage, a hanging system was made with the shell substratum, where they were separated by small pieces of PVC pipe (Figure 1). Culture conditions were as follows: temperature, 14 ± 2 °C; salinity, 32 ± 1‰; nutrients, Provasoli medium (Provasoli, 1963). The medium was changed every five days.

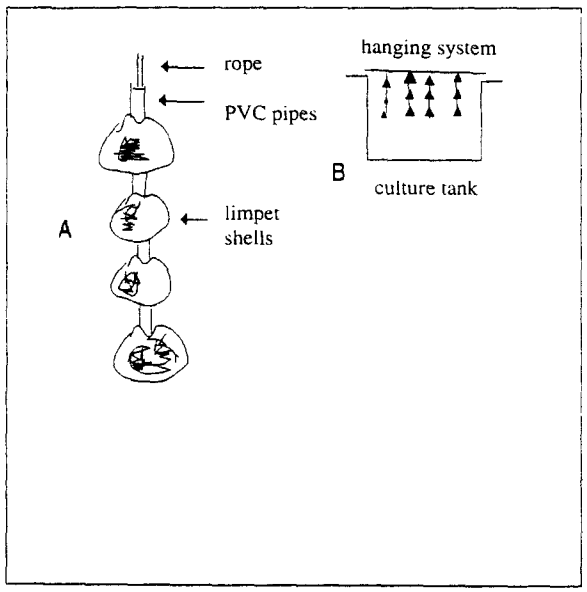

Figure 1. Hanging system used to culture *Gelidium rex* from spores and from re-attachment. A, detail of hanging system; B, placement of shells in culture tank.

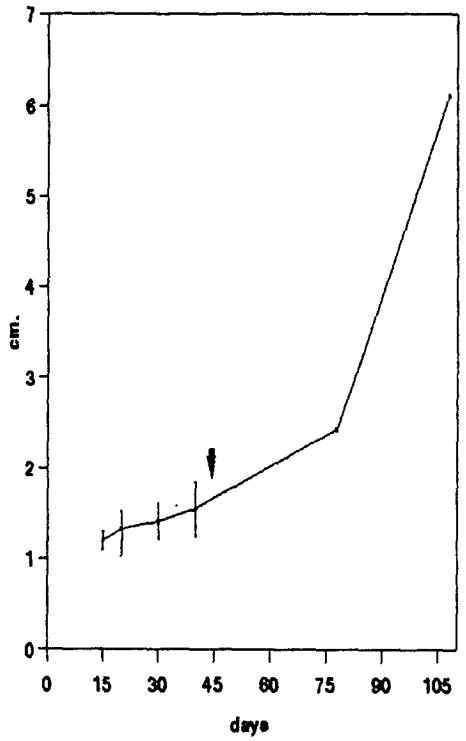

Figure 2. Growth of thalli of *Gelidium rex* from spores growing on limpet shells in tanks. Arrow indicates when thalli were transferred to the sea. Error bars represent standard deviations.

Culture from re-attachment

From the algae taken to the laboratory, 10 cm fronds were selected, placed over scallop shells (*Argopecten* sp.) and covered with plastic mesh, which was required to hold the algae against the substratum. The shells were hung in 0.6 ml tanks under the following conditions: temperature, 14 ± 2 °C; salinity, 32 ± 1‰, with constant and strong aeration and water circulation, with a sea water flow through rate of 20 l min^{-1}.

Outplanting

The substratum inoculated with spores was transferred to the sea 150 days after inoculation, and the substratum with re-attached algae, 78 days after the experiment began. The transfer was carried out at Bahía La Herradura, Coquimbo (29°58′00″ S, 71°22′00″ W), Chile. The shells were suspended from a long-line at a depth of 3 m.

Results

Culture from spores: laboratory phase

Average densities of 118 ± 4 spores mm^{-2} were obtained from the spore rain over the substratum, and the shells were homogeneously colonized. Thallus germination occurred in 24 hours, and the germination tube could be observed. Between six and 12 days, apical growth of the plantlets and the attachment of rhizoids to the substratum could be observed. After 79 days in culture, the plantlets showed an average size of 0.79 mm, and after 107 days, 1.05 mm, for a 3.3% growth rate per day. At the end of the laboratory phase of culture, thalli averaged 1.52 mm, representing a 1.5% growth rate per day (Table 1).

Outplanting phase

Thirty days after outplanting in the sea, thalli averaged 2.22 cm, and a significant increase in fouling on the shells and the thalli was observed. After 60 days, thalli averaged 4.15 cm (Figure 2), and they were strongly impaired by the fouling, mainly caused by balanoids.

The entire experiment was performed over six months, from substratum inoculation until two months after their transfer to the sea.

Table 1. Average length (in mm ± s.d.) and growth rate (% day^{-1}) of thalli of *Gelidium rex* cultured from spores. Period 1 is from day 79 to day 107, and period 2 is from day 107 to day 120.

Zone of shells in the hanging culture system	Average length			Growth Rate	
	Day 79	Day 107	Day 120	Period 1	Period 2
Upper	1.21 ± 0.01	1.71 ± 0.04	2.17 ± 0.10	2.47	1.70
Medium	0.65 ± 0.08	1.12 ± 0.21	1.44 ± 0.27	3.89	1.80
Lower	0.50 ± 0.11	0.82 ± 0.16	0.94 ± 0.09	3.53	0.97
Average	0.79 ± 0.31	1.05 ± 0.37	1.52 ± 0.51	1.49	

Culture from re-attachment: laboratory phase

Ten days after the experiment had begun, the thalli were completely attached, and the rhizoids were penetrating the substratum. Several plantlets growing from each thallus could also be observed. After 40 days the average size of the plantlets was 1.55 ± 0.30 cm.

Outplanting phase

Once the thalli were transferred to the sea on day, their growth was fast; 78 days after the culture had started their average size was 2.43 cm. Also, the development of trailing axes was observed, thus increasing their coverage of the substratum. The next observation took place on day 108, when the average size of the thalli was 6.10 cm (Figure 3). The presence of balanoids competing for space with the growing thalli was also observed on the shell surface.

In total, this experiment lasted three months, including the laboratory phase and transfer to the sea.

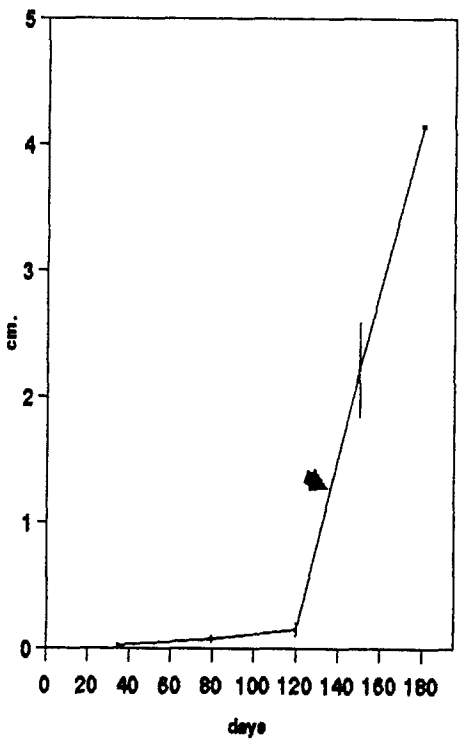

Figure 3. Growth of thalli of *Gelidium rex* from re-attachment of thalli to scallop shells in tanks. Arrow indicates when thalli were transferred to the sea. Error bars represent standard deviations.

Conclusions

Culture from spores produced good results. The sporulation technique presented no significant problems and was shown to be feasible to develop to a production level, assuring the required culture densities and the complete use of each substratum. In addition, the thalli obtained by this method showed a dramatic increase in their growth rate once they were transferred to the sea.

The re-attachment method is a good technique that can be applied at a production level, as it allows the selection and attachment of thalli with desired characteristics such as life history phase and/or better performance in growth. Moreover, the process can be performed at any season and regardless of the reproductive state of the thallus. Reattachment also makes it possible to use the apical portion of the thallus, avoiding major damage to natural fields. Undoubtedly, the most outstanding aspect of this technique is that it is faster than the method based on obtaining spores.

It must be noted that these are the first efforts made in this research. Much remains to be investigated to establish and develop the cultivation system in the sea.

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