Sporulation and sterilization method for axenic culture of *Gelidium canariensis*

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Abstract

A sporulation and sterilization procedure was used to establish axenic cultures of sporelings of *Gelidium canariensis*. Sporangial branchlets excised from the thallus were rinsed in distilled water twice and in 1% sodium hypochlorite (2 min). The branchlets were cultivated overnight in multiwell plates with 0.3 ml of autoclaved seawater to promote spore liberation in 90% of the cultivated branchlets. The branchlets were transferred to an antibiotic solution made of ampicillin, penicillin, rifampicin, nystatin (0.2 mg ml\(^{-1}\) each) and 0.1 g ml\(^{-1}\) of GeO\(_2\) in liquid PES for 45 days, during which clusters of spores (85–100 spores) were observed on the surface of the branchlet. After 55 days, they became axenic sporings with the prostrate and erect system characteristic of *Gelidium canariensis*. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Axenic culture; *Gelidium canariensis*; Rhodophyta; Sporelings

1. Introduction

Heterotrophic culture may provide a cost-effective large-scale alternative method for cultivation of algae that utilize organic carbon sources (Chen, 1996). Axenic heterotrophic cell and tissue cultures from macroalgae can be established from precultured axenic carposporings. For example, axenic heterotrophic cultures of the carragenophytic macroalgae *Grateloupia doryphora*, which produces secondary metabolites, sulfated polysaccharides and lipids, were successfully initiated from axenic spores (Garcia-Jimenez et al., 1996, 1998; Rodrigo and Robaina, 1997).

Axenic cultures of *Gelidium*, another important genus of macroalgae, producing over 50% of the agar and agarose world market (Armisen and Galatas, 1987), have not been established yet, although laboratory and field cultures of its spores have already been reported (Rodriguez, 1996, and Refs. therein; Rojas et al., 1996). Here, a sporulation and sterilization method for producing axenic sporings of *Gelidium canariensis* is described.
2. Materials and methods

Sporangial branchlets were excised from epiphyte-free thalli of *G. canariensis* collected at the North coast of Gran Canaria. Some branchlets were directly used to test the effect of the antibiotics according to the one-step antibiotic method (Saga and Sakai, 1982). As a result, we obtained an effective and non-inhibitory antibiotic solution mixture made of (final concentration in the culture medium): ampicillin (0.2 mg ml\(^{-1}\)), penicillin (0.2 mg ml\(^{-1}\)), rifampicin (0.2 mg ml\(^{-1}\)) nystatin (0.2 mg ml\(^{-1}\)) and germanium dioxide (0.1 g ml\(^{-1}\)).

To establish axenic cultures of sporelings we proceeded as follows: the branchlet was treated with 1% sodium hypochlorite for 2 min and then incubated overnight in 0.3 ml of sterilised seawater in multiwell plates. The seawater evaporated partially (between 30 and 50%), thus promoting a hydric stress for sporulation. The sporulation and sterilization procedure went on by transferring the branchlets to petri dishes with 20 ml of PES (Provasoli-enriched seawater; Provasoli, 1968) medium and the antibiotic solution described above. Controls of axenity were performed in bacteriological Marine Agar (Difco) to check the presence of surface contaminants, namely bacteria, fungi and other heterotrophic organisms. Thus, bacterial and fungal growth was not observed. Algal endophytes or epiphytes normally associated to *Gelidium* thalli were not recorded in the branchlets or sporelings.

3. Results and discussion

Sporulation was mainly observed when preceded by hydric stress. Ninety percent of all cultivated branchlets cultivated under hydric stress released spores, while in controls without hydric stress, only 40–50% of branchlets sporulated. The hydric stress overnight and rehydration of the branchlet in the medium with antibiotics seemed to push the spores out. The benefits of a hydric stress for sporulation have previously been reported in other algae (Edding et al., 1987). Clusters of axenic spores, attached to the branchlet in a mucous layer, were formed within 30–45 days after reculture (ca 85–100 spores per cluster. One or two clusters per branchlet (Fig. 1)).

The development of the spores of *G. canariensis* was similar to that described in other Gelidiales (Macler and West, 1987; Fredriksen and Rueness, 1989). The first divisions were observed 3 or 4 days after, when the spores settled in the culture dishes and formed new cells (germ tube), that developed a pigmented cell which originated an erect axis. Within 55 days, the latter became an incipient prostrate system with small buds along the axis (Fig. 2).

In conclusion, the sporulation induced by hydric stress and sterilization with an appropriate mixture of antibiotics made it possible to establish axenic cultures from spores of the agarophytic red seaweed *Gelidium canariensis*. It remains to investigate whether the spores will grow in media supplemented with organic carbon sources.

Acknowledgements

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Fig. 2. Germinated and axenic sporelings showing elongation of a bud (b) after 45 days in Petri dish culture. Scale bar = 0.2 mm.

References


