Aquaculture xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Short communication

The growth of alginate-encapsulated macroalgal spores

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ARTICLE INFO

Keywords:
Alginate
Ecklonia cava
Growth
Ulva intestinalis
Undaria pinnatifida

ABSTRACT

The present study examined the growth of alginate-encapsulated marine macroalgal spores of a green alga (*Ulva intestinalis*) and brown algae (*Undaria pinnatifida* and *Ecklonia cava*). We compared the initial germination and growth of gametes and thalli using alginate-encapsulated and non-encapsulated spores. Spores of the three algal species germinated easily; there were no significant differences between alginate-encapsulated and non-encapsulated spores ($p > 0.05$). After 45 days of culture, the alginate-encapsulated and non-encapsulated *U. intestinalis* spores were 35.315 ± 0.252 mm and 33.616 ± 0.815 mm in size, respectively. Encapsulated and non-encapsulated *U. pinnatifida* thalli (including gametophytes) were 24.928 ± 0.956 mm and 12.771 ± 0.458 mm, respectively. Encapsulated and non-encapsulated *E. cava* gametophytes were 648.35 ± 15.715 µm and 148. 33 ± 1.616 µm, respectively. Encapsulated spores of these three algal species tended to grow faster than non-encapsulated spores. Given these results, the artificial encapsulation with alginate was an effective way to enhance the growth of all three tested macroalgal spores.

1. Introduction

In nature, cryptobiotic organisms, including bacteria, microfungi, mosses, lichens, and blue-green algae, have dormancy states, which are a response to external stresses such as nutrient deprivation, desiccation, high temperatures, radiation, and caustic chemicals (Wood et al., 2013). In this context, the artificial encapsulation or immobilization of microorganisms is a current topic in biotechnology. Bacteria such as *Bacillus* and *Clostridium* protect against nutrient deficiencies by forming endospores, which are non-dividing, dormant bodies possessing a thin but tough proteinaceous coat (Henriques and Moran, 2000). Microorganisms such as yeast cells can be encapsulated within hard, thin shells using polymeric or natural materials to control immunosuppression or cell metabolism (Yang et al., 2009; Wang et al., 2011; Fakhrullin et al., 2009). Although most research has been directed toward bacteria, a recent trial has applied these techniques more widely, to organisms such as plant cells and microalgae (Zamani et al., 2012; Kandilian et al., 2017).

Studies focusing on microalgal encapsulation have found diverse applications, including stock culture management, nutrient and heavy metal removal in aquaculture, water quality control, wastewater treatment, and the production of secondary metabolites for biotechnological purposes (Chen, 2001; Moreno-Garrido et al., 2005; Solé and Matamoros, 2016). Like plants, algae have cell walls that protect cells from osmotic injury, exogenous catabolic enzymes, and other types of contamination (Sendbusch, 2007). For example, encapsulated eukaryotic microalgae (*Selenastrum capricornutum*) stored at a low temperature (4 °C) in darkness resumed normal growth after > 12 months of encapsulation (Faafeng et al., 1994). During encapsulation, algal cells maintain their respiratory and photosynthetic activities.

Encapsulation is performed by adding a natural polymer such as agar, agarose, carrageenan, chitosan, gelatin, collagen, or alginate (Kotwal and Shankar, 2009). Among these, calcium alginate is frequently used as a non-toxic matrix that permits the growth of different types of microorganisms (Smidsrød and Skja, 1990; Thepenier et al., 1985). The physiological stability of alginate depends on ambient ionic conditions; binding with cations such as Ca^{2+} stabilize the gel matrix for cell use (Robinson et al., 1985). Alginate encapsulation is also easier, cheaper, and more accessible than other methods (Lebeau and Robert, 2006).

Although many encapsulation studies have found success in small, single-celled prokaryotes and eukaryotes, few studies have been conducted on their application to macroalgae. Macroalgae are genetically diverse, structurally complex, and highly productive components of marine coastlines. They are generated from unicellular haploid spores, which differ considerably in the composition of their cell walls. For example, green algal cell walls consist mainly of glycoproteins, cellulose, pectins, and arabinogalactan proteins (Ciancia et al., 2012;

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https://doi.org/10.1016/j.aquaculture.2018.03.033
Received 30 November 2017; Received in revised form 14 March 2018; Accepted 19 March 2018
0044-8486/ © 2018 Published by Elsevier B.V.

Please cite this article as: Jung, S., Aquaculture (2018), https://doi.org/10.1016/j.aquaculture.2018.03.033

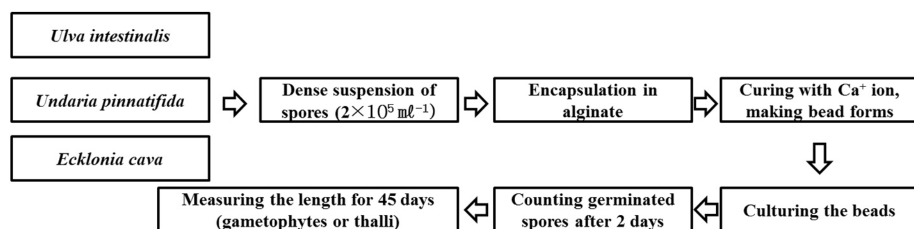

Fig. 1. Procedure for alginate encapsulation.

Estevez et al., 2009; Lahaye and Robic, 2007). Brown algal cell walls are composed of alginate, sulfated polysaccharides, and fucans (Michel et al., 2010), whereas those of red algae are composed of carrageenan and agarose (Whyte et al., 1985).

The objective of the present study was to observe the effects of alginate encapsulation on the germination and growth of three types of macroalgal spores: one species of green algae (*Ulva intestinalis*) and two species of brown algae (*Undaria pinnatifida* and *Ecklonia cava*).

2. Materials and methods

2.1. Reproductive cells

Reproductive thalli of *U. intestinalis* and *U. pinnatifida* were collected from the Pohang coastal region (36°09′14.8″N, 129°24′01.8″E); those of *E. cava* were obtained from the Busan coastal region (35°17′28.1″N, 129°16′05.9″E) in South Korea. They were transported to the laboratory in an ice box and then washed twice with distilled water to remove epiphytes and debris. The thalli of *U. intestinalis* and *E. cava* and sporophylls of *U. pinnatifida* were thoroughly washed in filtered seawater (0.45 μm filter), and spore liberation was facilitated as outlined by Fletcher (1989). Spore suspensions of each species were collected in beakers and their density adjusted to 2×10^5 spores mL^{-1} by adding filtered seawater (0.45 μm filter).

2.2. Encapsulation

We used sodium alginate (Sigma-Aldrich, St. Louis, MO, USA), extracted from brown algae, for encapsulation. We prepared 50 mL of 4% (*w/v*) sodium alginate and autoclaved it at 121 °C for 15 min. The alginate solution was then cooled to gelation at room temperature. We thoroughly mixed 50 mL of pre-counted spores with 50 mL of alginate solution to produce a final gel concentration of 2% (w/v) and cell density of 1×10^5 spores mL^{-1}. The algal spore-alginate solution was then transferred to a 1-mL pipette and 4.0 ± 1.5-mm beads were formed by extruding the mixture into a 3% (w/v) $CaCl_2$ seawater solution. By calculation, each bead contained 5×10^3 spores mL^{-1}. The beads were cured in 3% $CaCl_2$ seawater solution for 20 min and then washed three times with distilled water. Encapsulation was performed in the same manner for each macroalgal species.

2.3. Experimental design

For comparison, alginate-encapsulated and free spores were cultured in Provasoli's enriched seawater (PES) medium (Provasoli, 1968) for *U. intestinalis* and PES with iodine (PESI) medium (Tatewaki, 1966) for *U. pinnatifida* and *E. cava*. We cultured ten alginate-encapsulated beads in 100-mm diameter Petri dishes, and calculated a total spore density of 5×10^4 spores mL^{-1}. The same amount of free algal spores was inoculated into Petri dishes of the same size. The culture was illuminated at a light intensity of 55 μmol $m^{-2} s^{-1}$ using cool white fluorescent tubes in a light-dark cycle of 12 h/12 h, and the temperature was maintained at 25 ± 1 °C. We prepared ten replicates for each treatment. Samples from each treatment were collected after 2, 3, 6, 12, 15, 20, 25, 30, 35, 40, and 45 days. Growth was compared between the species by measuring gametophyte and thallus lengths on sampling days. The 2-day samples were observed for spore germination using a microscope (×200). From 3 to 45 days, we measured *U. intestinalis* thalli, *U. pinnatifida* gametophytes and thalli, and *E. cava* gametophytes (Fig. 1).

Table 1
Comparison of spore germination between encapsulated and non-encapsulated algae after 2 days of culture.

Species	Rate of spore germination (% dish^{-1})	
	Encapsulated	Non-encapsulated
Ulva intestinalis	58 ± 3.2	64 ± 2.8
Undaria pinnatifida	50 ± 1.1	48 ± 2.3
Ecklonia cava	26 ± 13.8	22 ± 3.1

2.4. Statistical analysis

All results are expressed as means ± standard deviation (SD), and significant differences were determined by paired Student's *t*-tests. We performed a two-way analysis of variance (ANOVA) to test for the interactive effects of species and encapsulation treatments on spore germination.

3. Results

Alginate-encapsulated and non-encapsulated spores of a green alga (*U. intestinalis*) and brown algae (*U. pinnatifida* and *E. cava*) were germinated following 48 h of culture. Table 1 shows the germination rates of the spores under each treatment. Non-encapsulated *U. intestinalis* spores had higher germination rates, but not significantly different from the rates of alginate-encapsulated spores ($p > 0.05$). Alginate-encapsulated spores of *U. pinnatifida* and *E. cava* had higher germination rates than non-encapsulated spores ($p > 0.05$). Fig. 2 shows digital images of the germination in encapsulated spores.

To observe the effect of encapsulation on growth, encapsulated and non-encapsulated spores were cultured for 45 days and their gametophytes and thallus lengths measured. The thalli of non-encapsulated *U. intestinalis* spores were longer than those of encapsulated spores after 6 days of culture. After 3 days of culture, the thalli lengths of encapsulated and non-encapsulated spores were significantly different ($p < 0.05$), at 0.062 ± 0.001 mm and 0.065 ± 0.001 mm, respectively. After 6 days of culture, the thallus length of non-encapsulated spores was 0.113 ± 0.002 mm, which was not significantly different from that of encapsulated spores, 0.112 ± 0.005 mm. From 9 to 45 days of culture, the growth of encapsulated spores was significantly higher ($p < 0.05$) than that of non-encapsulated spores. After 45 days of culture, the alginate-encapsulated and non-encapsulated *U. intestinalis* spores were 35.315 ± 0.252 mm and 33.616 ± 0.815 mm in size, respectively (Fig. 3).

Because the life cycles of *U. pinnatifida* and *E. cava* are longer than that of *U. intestinalis*, we measured the gametophyte length in this species as a proxy for growth. The gametophytes of encapsulated *U. pinnatifida* spores were longer than those of non-encapsulated spores between 3 and 45 days of culture. The gametophyte of this species retained its shape until 20 days of culture, and then developed thalli.

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