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SCIENCE DIRECT
Aquaculture 254 (2006) 219–226
Aquaculture
www.elsevier.com/locate/aqua-online

Formation and early development of tetraspores of *Gracilaria lemaneiformis* (Gracilaria, Gracilariaceae) under laboratory conditions

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Received 24 March 2005; received in revised form 17 August 2005; accepted 19 August 2005

Abstract

Morphological and culture studies of tetraspores of *Gracilaria lemaneiformis* were carried out under laboratory conditions. Relationships of germination rate, diameter and survival rate of tetraspores from 1st generation branches with grads of temperature and irradiance were determined, respectively. The result showed that 1st generation branches is in the majority of the tetraspores shedding and tetraspores from which had highest survival rates than other parts of the sporophytic plant. The time tetraspores used developing from giant unicells to diads, which both existed on the epidermis, then to tetraspores off the matrix, was only approximately 3 weeks all through. However, tetraspores spent more than two months developing into germlings of gametophytes. It was shown that temperature variation (10, 15, 25, 30 °C) with the light of 30 μmol m^{-2} s^{-1} had significant effects on the germination rate and diameter, but had no apparent effect on survival rate (ANOVA, $P<0.01$). Germination rates of tetraspores reached the maximum at 20 °C, which was significantly higher than those at other temperature levels ($P<0.01$), whereas 15 °C seemed to be optimal temperature for the diameter. All the three growth parameters (germination rate, diameter and survival rate) yield highly significant variations with irradiance treatments at room temperature (ANOVA, $P<0.01$). The optimal germination rate was detected at the irradiance of 30 μmol m^{-2} s^{-1} ($P<0.01$). The photon flux density which exceeds 480 μmol m^{-2} s^{-1} have apparently negative effect on diameter and survival rate.

Keywords: *Gracilaria lemaneiformis*; Tetraspore; Gametophyte; Survival rate; Photon flux density

1. Introduction

Gracilaria spp. (Rhodophyta) are important economic algae used for agar extraction (Marinho-Soriano, 2001; Marinho-Soriano and Bourret, 2003; Freile-Pelegrín and Murano, 2005), natural products with important bioactivity (Mazumder et al., 2002; Melo et al., 2002), edible market seaweed in some parts of the world (Santelices and Doty, 1989; Norziah and Ching, 2000), food binder for aquaculture (Peñaflorida and Golez, 1996), as well as efficient heavy metal container (Sfriso et al., 1994).

In the past, most of the *Gracilaria* harvest comes from wild stocks as the marine aquaculture only accounts for a little part of the total biomass. By 1991 approximately one-third of the harvest was from cultured sources of *Graciliaria* (McHugh, 1991; Glenn

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doi:10.1016/j.aquaculture.2005.08.026

et al., 1996). Recently, most of the culture methods rely on vegetative fragments, rather than carpospore or tetraspores of mature thalli, as the propagating units to obtain new plants (LaPointe et al., 1976; Santelices and Doty, 1989; Hurtado-Ponce, 1990; Trono, 1990; Bravo et al., 1992; Friendlander, 1992; Hurtado-Ponce et al., 1992; Nelson et al., 2001; Ryder et al., 2004). However, practices prove that thalli farming have many disadvantages: many species are too small and delicate to be efficiently grown by hand-planting; for the larger species, vegetative propagation is inefficient because it requires large amounts of propagating material to start or revitalize mass plantings (Glenn et al., 1996); in small-scale culture, for instance, 20–30% of the harvest may be used as seed material (Hurtado-Ponce et al., 1992). Scientists have pointed out the need for mass-production, spore-culture method for *Gracilaria* similar to those used for other seaweeds (Lin et al., 1979; Buschmann and Kuschel, 1988; Romo-Donoso, 1988; Levy et al., 1990; Glenn et al., 1996; 1998).

Being one of the mini-phases of the life history of *Gracilaria*, tetraspores connect phases of sporophytes and gametophytesa. However, differing from carpospores, tetraspores are rarely used as seeds to propagate the important economic marine algae and the information about the development of the tetraspores is still limited. The development of new cultivation methods for *Gracilaria* requires control over reproduction and hence knowledge of its life history. In this paper, we used sporophyte thalli of *Gracilaria lemaneiformis* as the matrix to study the morphologic changes of tetraspores and the effect of extra environmental factors, irradiance and temperature, on the survival of tetraspores during their early developing period.

2. Materials and methods

Reproductive mature cystocarpic and tetrasporic of *G. lemaneiformis* were collected in Oct. 2004 from the intertidal zone of Zhanshan, Qingdao, China. Fertile tetrasporophytic fronds were recognisable by the presence of tetrasporangia scattered over the whole fronds forming reddish marbling with an invert light microscope (ZEISS, Axiovert 135 M, Germany). The fertile tetrasporophytic fronds were selected and rinsed in distilled water twice and in 1% sodium hypochlorite for 2 min. Then approximately 50 g of fertile fronds of each phase were kept in plastic containers, each containing 500 ml of enriched seawater with ES (McLachlan, 1979) under 30 μmol m^{-2} s^{-1}, 20 °C and with a photonperiod of 12 : 12 h LD.

2.1. Treatment of thalli used for tetraspores release

Magnification of a microscope revealed that most of the giant unicell of pre-tetraspores were distributed on the epidermis of 1st generation branches of *G. lemaneiformis* and the subsequent experiment also showed that tetraspores from 1st generation branches had the highest survival rate, so the 1st generation branches of sporophytic of tetrasporophytic fronds were cut (about 5 cm in length) and used for the follow experiment. 2nd and 3rd generation branches were also cut in the same length for the determination of survival rates of tetraspores (Figs. 1 and 2).

It is said that some bacteria can inhibit algal spore germination (Egan et al., 2001), so the branchlets used for the experiment were all rinsed with antibiotic enriched seawater solution made of ampicillin, penicillin, rifampicin, nystatin (0.2 mg ml^{-1} each) and 0.1 g ml^{-1} of GeO_2 for about 6 h before used.

2.2. Morphological changes and survival of tetraspores during their development process

Samples of slice containing tetraspores at different developing phases from 1st generation branches were obtained at intervals using a surgical blade (Sigma) to slice the branchlet squeezed firmly in the middle of two slides, then the sample was observed with a light microscope (Nikon, ECLIPSE 50i, Japan). Tetraspores off epidermis from all types of branches were observed with a light microscope (ZEISS, HBO 50, Germany) with or without fluorescent light.

Branchlets of different colonies used for the determination of tetraspores distribution were observed directly under an inverted light microscope (ZEISS, Axiovert 135 M, Germany) at the magnification of ×200. 20 eyeshots were selected randomly and all the tetraspores in which were all included.

Every Petri dish contained the same type of 15 branchlets, and all branchlets were cultured under 15 μmol m^{-2} s^{-1}, 15 °C and a photonperiod of 12 : 12 h LD in enriched seawater.

2.3. Effect of temperature and photon flux density upon tetraspores

The Petri dishes containing branchlets were cultured in cultivation chambers. Temperature treatment were performed at 5 temperature grads (10, 15, 20, 25, 30 °C) under light of 30 μmol m^{-2} s^{-1} provided by cool-white fluorescent tubes of 40 W. Photon flux density treatments were performed by culturing sets of spores