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Aquaculture 148 (1997) 77–83

Aquaculture

Mass cultivation of the agar-producing alga *Gracilaria chilensis* (Rhodophyta) from spores

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Accepted 27 July 1996

Abstract

Gracilaria chilensis has been extensively cultivated in Chile by means of vegetative propagation. This method consists of outplanting pieces of thalli, which are anchored by several methods to muddy or sandy substrata at densities of about 1 kg (wet weight) m^{-2}. Here we describe a new technique for mass cultivation of *Gracilaria* on ropes inoculated with spores. The paper describes: (i) induction of sporulation and fixation on polypropylene ropes; (ii) evaluation of spore settlement on the ropes; (iii) incubation of the germlings in tanks with agricultural fertilizers and (iv) outplanting of the ropes with plantlets to a pilot stand of about 5000 m^2 in an estuary in Central Chile. The technique will allow the establishment of a commercial farm using the stock of spores contained in 30–40 kg cystocarpic *Gracilaria chilensis* ha^{-1}, compared with the 10000 kg of seaweed necessary for planting 1 ha using the traditional methodology.

Keywords: *Gracilaria chilensis*; Farming; Culture methods; Management; Mariculture

1. Introduction

Since 1950 the agarophyte *Gracilaria chilensis* Bird, McLachlan and Oliveira has been exploited in Chile, and in recent years the increasing demand for agar has greatly contributed to the depletion of the wild populations (Pizarro, 1986; Alveal, 1988a,b). In 1982 commercial cultivation of *G. chilensis* began in Chile (Ponce, 1988) in response to overexploitation of the wild stocks, and by 1991, 84% (ca. 60000 t wet weight) of the production was obtained from cultivation (Avila and Seguel, 1993). Chilean commercial cultivation has been based on the bottom planting of vegetative material (Pizarro, 1986; Alveal, 1988a; Westermeier et al., 1988; Santelices and Doty, 1989). In Malaysia,

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PII S0044-8486(96)01415-9

however, spores attached to rope have been utilized on a pilot scale to produce different species of *Gracilaria* (Doty and Fisher, 1987). Here we describe a successful pilot study, utilizing spores, for the commercial cultivation of *G. chilensis*.

2. Materials and methods

2.1. Collection and maintenance of reproductive plants

Mature carposporic plants were collected in the Tubul estuary, central Chile (37°14′S, 73°26′W), drained to remove excess water and taken to the laboratory, in darkness, in thermally insulated containers (15 ± 2°C). The plants were washed with seawater in order to remove sand and mud, then cleaned of visible epiphytes. Taking into account the high resistance of *G. chilensis* to the osmotic changes, they were briefly rinsed with tap water and lastly rinsed with sterile seawater in order to promote the spore release from the cystocarps.

2.2. Inoculation of substrata

Fertile cystocarpic plants (200 g) were put into a 340-l tank with filtered seawater (0.45 μm) set up on 32 ropes, each 5 m long, as shown in Fig. 1(B). The algae were kept at 20 cm above the ropes to ensure a uniform distribution of the spores after sporulation. The spores were left for 24 h, in dim light, to attach to the 3–4-mm polypropylene ropes wrapped around a plastic frame. Spore release was checked on five slides placed on the frames with the ropes, one in the center and the others in each corner of the frame. Spore settling was quantified by counting the total number of cells

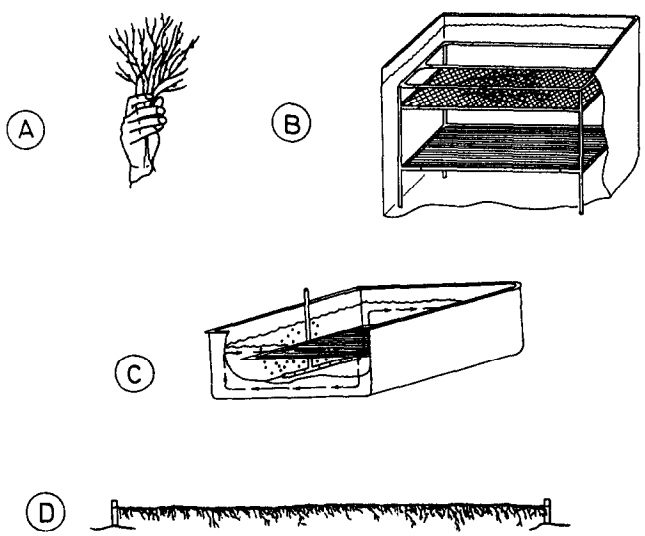

Fig. 1. Schematic view of the process of *Gracilaria chilensis* cultivation using spores. (A) Branch of fertile frond; (B) tanks with an upper screen with the fertile fronds, and a lower frame with the ropes that will receive the spores; (C) aerated tank for incubation of sporelings; (D) rope with *G. chilensis* growing in the field.

in 50 areas on each slide. During the following day, the frames with ropes were inverted and inoculated with a fresh stock of reproductive material. This methodology was performed on 32 ropes, each 5 m long (160 lineal meters of rope).

2.3. Incubation in tanks

The incubation was performed in a greenhouse in 150-l tanks with filtered seawater, enriched with a mixture of agricultural fertilizers in concentrations of 0.06 g l^{-1} for sodium nitrate and 0.01 g l^{-1} for sodium phosphate. The tanks were kept at 15–20°C, ambient light, 28–32‰ salinity and 7.2–8.2 pH; water movement was provided by bubbling air. The incubation lasted for 2 months by which time the microthalli attained a height of 0.7–1.5 mm (Fig. 1(C)).

2.4. Outplanting in the field

The 32 ropes measuring 160 m in length, with the attached microthalli, were transferred to the Raqui estuary (37°14′S, 73°26′W), in thermally insulated containers (15 ± 2°C). The ropes were held between poles driven into the sandy substratum, 30 cm above the bottom, 1 m from each other and 70 cm below the low water spring tide, in rows parallel to the direction of the current (Fig. 1(D)). Growth measurements were carried out on five ropes randomly selected out of the 32 ropes outplanted to the field. Growth was monitored monthly from September 1990 until April 1991, by determining the drained fresh weight of each one of five selected ropes.

3. Results

3.1. Inoculation of substrata and incubation in tanks

The spores shed by 200 g of mature plant allowed the settlement of at least 400 spores cm^2 as counted on the slides placed on the frames (Table 1). However, 200 spores cm^{-2} appeared adequate to avoid overdensity and competition for light and nutrients between the germlings. The polypropylene multifilament was a substratum to which the spores attached firmly. No detachment could be seen when bubbling of air was started. Fouling organisms such as diatoms, *Ulva* and *Enteromorpha*, were conspicuous from about 3 weeks after inoculation. Contamination was especially noticeable

Table 1
Average density of spores released from cystocarpic plants of *Gracilaria chilensis* on slides placed on frames with ropes, from September 1990 to April 1991 (n = number of measurements to estimate average density of spores)

	Rope 1	Rope 2	Rope 3	Rope 4	Rope 5
Average density of spores per cm^2	469	430	405	409	554
Standard deviation	466	558	368	329	355
n	250	250	250	250	250

Fig. 2. *Gracilaria chilensis* sporelings growing on a rope filament.

during the summer but was considerably lessened by cleaning the ropes with a soft brush and reducing the light with plastic screens. After 2 months of incubation the germlings attained 0.7–1.5 mm height and were outplanted in the field (Fig. 2).

3.2. Growth in the field

The ropes with the attached germlings were outplanted to the estuary in January 1990. The germlings exhibited sustained growth over 9 months and, in September 1990, attained an average density of 2.5 kg (s.d. $\pm$ 1.10) m^{-1} of rope. During December, the average density with extreme values between 2 to 15 kg m^{-1} was 6.5 kg m^{-1}, and the

Fig. 3. Rope with *Gracilaria chilensis* grown from spores after 12 months in the field with a density of about 6 kg per lineal meter.

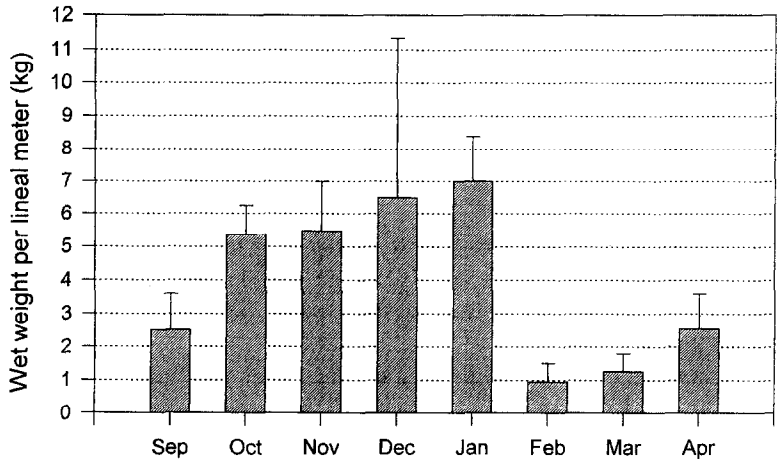

Fig. 4. Biomass variation (wet weight) and standard deviation of *Gracilaria chilensis* per meter of rope.

high variability of the measurement (s.d. ± 4.84) was due to the detachment of the heaviest plants. A peak of 7 kg (s.d. ± 1.39) was reached in January (Fig. 3), but density on ropes decreased drastically in February (average density 0.95 kg s.d. ± 0.55) (Fig. 4). The vigorous growth of *G. chilensis* during January resulted in the increased detachment of some plants from the ropes in the following months due to the tidal currents, suggesting that the maximum weight of plants on the ropes should not exceed 7 kg wet weight m^{-1}. About 30 to 67% of the ropes showed higher densities than their monthly average.

4. Discussion

The traditional methods used in commercial *Gracilaria* farms in Chile reported by Pizarro and Barrales (1986), Westermeier et al. (1988), Santelices and Doty (1989), Avila and Seguel (1993) and Buschman et al. (1995) have been based on the use of 0.5–1.0-kg wet weight fragments which are directly buried in the soft bottom in rows or tied to stones or polyethylene tubes filled up with sand. The success of such methods has been due to four biological attributes of the species. (i) The terete-branched morphology of the plants allows rapid regrowth even though plants are covered by sediment, which prevents them from being dislodged by underwater turbulence. (ii) *Gracilaria chilensis* tolerates a broad range of salinity (ca. 7–37‰ S) as reported for *Gracilaria* strains from Maullin river (VA strains), Chile (47°37′S) (Bird and McLachlan, 1986), as well as a broad range of temperature (7–28°C), as reported for VA strains and *Gracilaria* strains from Coquimbo Bay (COQ strain), Chile (29°56′S) (McLachlan and Bird, 1984). (iii) The species is tolerant to gentle desiccation which allows it to maintain its viability during the handling procedures required to anchor them to the bottom. (iv) *Gracilaria chilensis* shows a very high growth rate. Pizarro and Barrales (1986), in experimental studies carried out in northern Chile, showed that an initial biomass of 450 g m^{-2} produced an extrapolated yield of 126 t ha^{-1} over 6 months (spring and early summer).

Some commercial yields in some very productive farms in Chile can reach values of about 100 t $year^{-1}$ ha^{-1} during the first or second year after planting (R. Rojas, personal communication, 1993). The physiological attributes can explain the success of the transplantation procedures of commercial stocks from estuaries to marine farms and vice versa and from southern sites (Chiloe Island, 42°S), with both broad salinity and temperature ranges, to Coquimbo Bay with higher and more constant hydrographic conditions.

Three main constraints remain unsolved for better crop management: (i) ageing of the thalli, (ii) occurrence of epiphytes and (iii) high amount of seaweed in the initial seedling stock. The procedures here described could be a useful tool to solve or ameliorate such problems.

Recently, Buschman et al. (1995) pointed out that, after planting, a site can be highly productive for 2 or 3 years, but subsequently the production drops rapidly. This performance has been interpreted as due to the ageing of the thalli. *Gracilaria* has an active apical meristem which is removed during each harvest period leaving the older parts of the thalli behind. Consequently, ropes with new plants growing up from spores could be quickly installed in the farm ground in order to replace old stands, once the drop in production occurs.

The heavy seasonal occurrence of epiphytes, such as seaweeds and diatoms, is a critical problem that could be associated with the loss of chemical defenses by old plants. Matamala and Sanhueza (1988), Kuschel and Buschman (1991) and Westermeier and Rivera (1991) report on the dynamics of epiphytic populations on natural and cultivated beds of *Gracilaria*. Kuschel and Buschman (1991) give values as high as 400% epiphytism (g epiphyte g^{-1} *Gracilaria*) for intertidal experimental plots in southern Chile, and Gonzalez et al. (1993) suggest that the high harvest frequency could be one of the several mechanisms (such as changes of temperature, increasing light and water motion) contributing to the epiphyte outbreaks. In many cases the supply of new stocks of young plants arising from spores could be employed for maintaining the vigor of a site.

A third important consideration is related to the investment in the initial seedling stock. With the above-described technique, 400 g of algae is necessary for the inoculation of about 100 m of 3-mm-diameter rope in each frame. Extrapolating these results, the spores contained in about 40 kg of cystocarpic *Gracilaria* will be necessary for planting 1 ha (or 10000 m of inoculated ropes, equivalent). On the other hand, the cost of *Gracilaria* for the establishment of 1 ha by vegetative propagation fluctuates between US$1020 and US$2040 (US$0.17 kg^{-1} wet algae) for densities of 0.6 to 1.2 kg m^{-2} given by Buschman et al. (1995). The approach and results of this project coincide with those reported by Doty and Fisher (1987) and those summarized for Santelices and Doty (1989) for the farmability of Penang *Gracilaria* species, in Malaysia.

Spore culture is not expensive as a vegetative method of cultivation. The equipment needed for spore cultivation is simple and can easily be utilized by fishermen cooperatives. Since 1992, this technique has been adopted very successfully by Algas Marinas S.A., a commercial farm in southern Chile, in a program for the replacement of their old crops by new stands of plants growing out of plants from spores (R. Rojas, personal communication, 1994).

Furthermore, with this method it could be possible to grow diploid plants, haploid plants or a combination of both phases which may be an important application for the management of the species. We expect that this approach can also be employed with success using other agarophytes, similar to that achieved with *Porphyra*, brown and green algae.

Acknowledgements

Support for this study was provided by the United Nations Development Program (PNUD), Algas Marinas S.A. (Chile) and the University of Concepcion, Chile. The technical assistance of Mr. Mario Nuñez (IFOP, Chile) is gratefully acknowledged as well as the help of Dr. Fergus Molloy and Ms. Julia Ramirez for correction of the text. Thanks are also due to the anonymous reviewers for making improvements on the contents of this paper and for checking the English manuscript.

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