The Effect of Some Environmental Factors on Biomass and Agar Content of *Gracilaria corticata* (Gracilariales, Rhodophyta)

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Received: 2016-09-25 Revised and accepted: 2017-05-28

Abstract

The effects of some environmental parameters on biomass and agar yield of *Gracilaria corticata* were examined under laboratory conditions. The macroalga was collected from natural tidal pool with in Bostaneh coast (the Persian Gulf) in May 2004. In the laboratory, biomass and agar yield were measured after 45 days examining effect of different temperatures (24, 27 and 32°C), photon irradiances (24, 66 and 94 photons $m^{-2}s^{-1}$), and concentrations of ammonium phosphate [unenriched seawater (0), 0.04 and 0.08 gl^{-1}] in culture. Except for concentration of ammonium phosphate, other environmental variables showed no significant relationship ($P>0.05$) with biomass and agar content of *G. corticata*. The agar yield derived from the alga cultivated at 0.08 gl^{-1} ammonium phosphate (22.40±1.81%) was significantly greater than the other treatments that makes *G. corticata* one of the commercial agarophytes ($p<0.05$).

Keywords: Biomass, Temperature, Irradiance, *Gracilaria corticata*, Ammonium phosphate, Agar.

Introduction

The red algal genus *Gracilaria* is harvested and cultured commercially in many countries because of its economic importance in producing agar. It is used as a source of traditional seaweed salad and also fed to shellfish (abalone) in many countries (Kakita and Kamishima, 2006). Agar consists of two main fractions: A neutral polymer, agarose of high gel strength and agaro-pectin, a sulfated polysaccharide of low gel strength. It has a large range of usage such as food elements, cosmetic products and pharmaceuticals. The largest amount (65%) of agar produced in the world is derived from *Gracilaria* species (Orduna-Rojas et al., 2008). Environmental factors play an important role in growth, reproduction and distribution of marine algae. There has been researches for evaluation of the effect of temperature (Engeldow and Bolton, 1992; Tsai et al., 2005; Choi et al., 2006) light (Molloy, 1992) and nutrients (Hanisak, 1990; Yang et al., 2006) on biomass of *Gracilaria* species. Several studies have shown

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that agar yields depend on species, environmental parameters (Orduna-Rojas et al., 2008), season (Marinho–Soriano and Bourret, 2003) and method of extraction. In order to culture this valuable alga, we need to recognize the best conditions for its growth and agar yield.

There are 16 species of *Gracilaria* in Iranian waters, among them *Gracilaria corticata* is one of the most abundant species. Studies on seasonal variation of biomass conducted by (Stellaroslin, 2001a) and agar content (Stellaroslin, 2001b) in India, biomass and amount of agar in Iran (Rafiee et al., 2005), effects of some growth regulators (Subbaraju et al., 1981), pH and salinity (Singh et al., 1979) on *G. corticata* have been carried out so far. According to the authors' searches to date, there is no published report about the effect of irradiance, temperature and nutrients, under controlled conditions, on biomass and agar yield of this alga.

The objective of this study was to determine variations of length, biomass and agar yield under a range of temperatures, irradiances and concentrations of ammonium phosphate in laboratory. This study provided some preliminary information required to improve growth and agar for the potential cultivation of this agarophyte.

Materials and Methods

The Bostaneh coast has two tidal pools with 0.5 meter average depth at low tide among a rocky platform (Fig. 1). The substrate of pool consists of sand and small stones, where the macro algae are attached. The algae are found along the grooves between the rocks in platform too. The macro algae do not become exposed during low tide.

Sampling of *G. corticata* was performed in May 2004 on the Bostaneh coast (26°31'N, 54°50'E), in the Persian gulf.

After harvesting, the macroalgae were washed with seawater and removed epiphytes. Apical segments (5cm) were cut from the thalli and cleaned with a soft brush. For each treatment (aquarium), 15 fresh segments were inoculated into two 144L aquarium with diameters of 40×90×40cm. Only healthy thalli were selected for experiments. In this study, the suspension method was used for culture (Westermeier et al., 1993). The plants were attached with nylon ties to three nylon ropes suspended along the length of aquarium at a depth of 5cm (Nelson et al., 2001). To avoid penetration to outside light, the aquariums were covered by aluminum foil cultures were incubated in 16:8h light:dark photo cycle using cool-white fluorescent lamps and salinity of 38±2 mgl^{-1}. Water was refreshed weekly with natural sea water. The algae were harvested after 45 days in all treatments. The biomass (weight of all thalli) of thalli were measured after 20 and 45 days.

Three culture conditions were considered. First, three levels of temperature (24, 27, 32°C) were examined while other factors kept constant: 94 photons $m^{-2}s^{-1}$ for irradiance and seawater without adding nutrient for medium. Second, three variations of irradiances (24, 66 and 94 photons $m^{-2}s^{-1}$) were tested considering 24°C for temperature and seawater without adding nutrient for medium. Third, three concentrations of ammonium phosphate [unenriched seawater (0), 0.04 and 0.08 gl^{-1}] were considered for nutrient treatments keeping temperature and irradiance to 24°C and 94 photons $m^{-2}s^{-1}$, respectively. The temperatures provided by aquarium heater and

the ammonium phosphate was placed weekly (Israel et al.,1999). In all treatments the medium was vigorously aerated by about 3L air min^{-1} (Mercado et al., 2000).

Extraction of agar was performed according to (Freile-Pelegrin, 2000) with some modifications. *G. corticata* were collected from natural population, and from the irradiance, temperature and nutrient experiments dried under shadow and then milled. Alkali treatment was used for this mean. Prior to extraction, dried algae were treated with 62.5ml of 1M NaOH and the whole volume was reached to 500 ml by adding distilled water. Then the suspension was stirred well by a glass stirrer on Ben Marry bath at 80°C for 2 hours. Samples were filtered and adjusted to pH=6 by washing with distilled water at 60°C and adding sulfuric acid. Then, samples were heated for one hour at 30 pound in 2 inch pressure in an autoclave. The heated algae filtered through cotton tissue and were allowed to gel at room temperature for 3 to 4 days and dried. The agar yield was calculated as the percentage of dry matter.

Fig. 1. Map of the Persian Gulf. The arrow shows sampling site (about 200km west of Bandar Abbas)

The data were analyzed using one way ANOVA. Turkey's comparison test was conducted to distinguish significantly different results. (Wakibia et al., 2001; Kakita and Kamishima, 2006). P-values less than 0.05 were considered significant.

Results

In temperature treatments, biomass obtained at 32 that increased from (day 0-45) 14.53±0.3 to 24.91±16.6 g, respectively (Fig. 2). The greatest amount of biomass of irradiance treatments observed at 66 photons $m^{-2}s^{-1}$) with 41.93±15.79 to 70.93±10.55 g, respectively (Fig. 3). The biomass and agar content of macroalga cultivated under various irradiances and temperatures showed no significant difference between treatments ($P<0.05$). Biomass and agar content of *G. corticata* were significantly different between levels of ammonium phosphate treated. The highest biomass obtained at concentration of 0.04 gl^{-1} ammonium phosphate with 52.31±9.48 g (Figure 4). The agar yield of *G. corticata* at 0.08 gl^{-1} ammonium phosphate with amount of 22.97±1.81% was significantly greater than the highest agar extracted from temperature (15.32±0.97%) and

Table 1. Effect of concentration of ammonium Phosphate, temperature and irradiance treatments on agar content (percentage of dry weight) of *G. corticata* after 45 days culture . (Mean± SD)

	Ammonium Phosphate (grl^{-1})		
	0	0.04	0.08
Agar%	11.99±2.99	16.38±1.35	22.97±1.81
	Temperature (°C)		
	24	27	32
Agar%	15.32±0.97	14.07±0.66	13.9±0.56
	Irradiance (µmol photons $m^{-2}s^{-1}$)		
	24	66	94
Agar%	12.98±2.59	12.10±3.52	9.59±8.1

irradiance (12.98±2.89%) treatments (Table 1).

Discussion

Agar yield was significantly (p=0.031) correlated with biomass. Correlation between biomass and agar yield appears to be species dependent (Givernaud et al., 1999). Our data supports the works of Marinho-Soriano et al. (1999, 2001) who found a higher biomass and agar yield of *G. bursa* pastoris during the summer and lower yield in the winter. There were an inverse relationship between biomass and agar content of *G. canarensis* in Spain and *G. multipartitain* Morocco (Givernaud et al., 1999) and *Gracilaria* sp. (Wakibia et al., 2001). Biomass and agar content of *G. corticata* were significantly different between levels of ammonium phosphate treated. The highest biomass obtained at concentration of 0.04 gl^{-1} ammonium phosphate with 52.31±9.48g. The agar yield of *G. corticata* at 0.08 gl^{-1} ammonium phosphate with amount of 22.97± 1.81% was greater than the highest derived from natural population on August (10.01± 0.02%) (Rafiee et al., 2005).

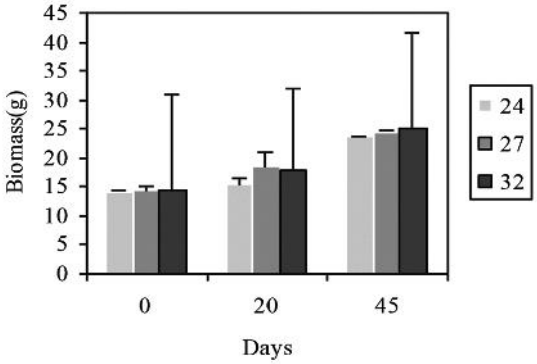

Fig. 2. Effect of temperature treatments on biomass (g) of *G. corticata* over 45 days culture. (Mean±SD)

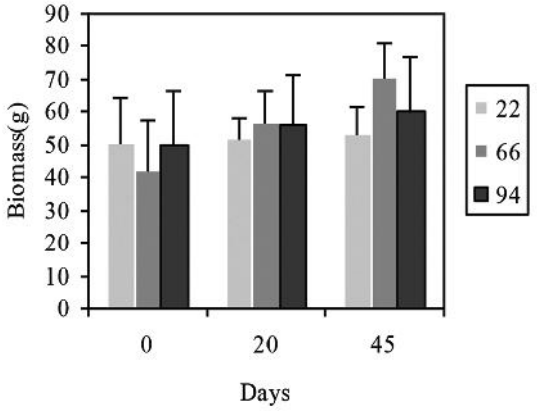

Fig. 3. Effect of irradiance treatments on biomass (g) of *G. corticata* over 45 days culture. (Mean±SD)

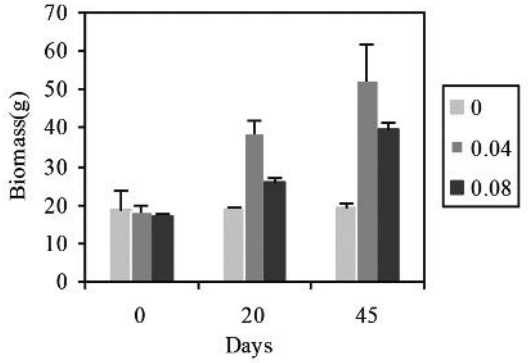

Fig. 4. Effect of ammonium phosphate treatments on biomass (g) of *G. corticata* over 45 days culture. (Mean±SD)

The biomass and agar of macroalga were not affected by variations of temperatures and photon irradiances. Unlike the other reports (Kakita and Kamishima, 2006), our study was similar to *Gracilaria sp*. in South Africa (Wakibia et al., 2001) and *G. coronopifolia* (Tsai et al., 2005). It has been reported that *G. vermoculophylla* can tolerate a wide range of 5 to 30°C and maximum growth were to be at 15-20°C. Similar results have been observed in *G. lemaneiformis*, *G. tikvehiae*, *G. chilensis*, *G. vermiculophylla*, *G. verrucosa* and *G. chorda* that growing well in temperature as high as 30°C (*Yokoya* et al., 1999; Choi et al., 2006). McLachlan and Bird (1986) found that *Gracilaria* species that tolerated 30°C or higher were from warm water habitats. Similarly, in this study *G. corticata* grew well in temperature higher than 30°C (32°C). In the field study *G. corticata* showed suitable growth at 32 and

34°C in June, August and July, respectively (Rafiee et al., 2005).

There are numerous reports that irradiance significantly influences the growth of *Gracilaria*, (e.g, Engeldow and Bolton, 1992; Molloy, 1992; Kakita and Kamishima, 2006).

Irradiance levels between 50 photons $m^{-2}s^{-1}$ and in excess of 1450 photons $m^{-2}s^{-1}$ have been reported for maximum growth of *Gracilaria* (Friendlander et al., 1987; Lingell et al., 1987). Since the irradiances tested in this study were in this range, no significant difference in biomass was found. Similarly to our findings, *G. coronopifolia* has not been affected by irradiance (Tsai et al., 2005).

The major factors that significantly influenced biomass and agar content of *G. corticata* were concentrations of ammonium phosphate ($P< 0.05$)As obtained from the previous field study concentrations of phosphate had no significant effect on growth and agar of *G. corticata* ($P=0.70$, $P=0.36$) and ammonium had positive effect. It seems that the factor in nutrient treatments affected growth and agar yield was ammonium.

There have been mixed reports regarding the role of nitrogen in the growth of *Gracilaria*. Bird et al. (1981) reported increased growth with the addition of ammonium or nitrate. Lapointe and Ryther (1978) reported higher growth rates of *G. foliifera* after fertilization with ammonium compared to fertilization with nitrate, which is similar for *G. gracilis* in South Africa (Smith et al., 1997), *G. parvispora* in Hawaii (Glenn et al., 1999) and *G. coronopifolia* in southern Taiwan (Tsai et al., 2005). Similarly, enrichment experiments have been conducted with *G. foliifera* (Deboer et al., 1978) which responded positively to ammonia enrichment up to 5 $mmolm^{-3}$. Also, *G. salicornia* responded positively to the addition of 6 $mmolm^{-3}$ ammonia to unenriched seawater in Kaneohe Bay (Larned, 1998). In the laboratory experiments, (fish and seaweed culture systems).

Yang et al. (2006) demonstrated that *G. lemaneiformis* was able to remove inorganic nutrients effectively and the concentration of NH_4^+–N decreased by 85.53% after treatment.

Thalli of *Gracilaria* have been shown to be capable of rapid exploitation of ammonium in the environment by diffusive and active uptake (D'elia and Deboer, 1978). Uptake is followed by storage of the nitrogen to facilitate growth later, under nutrient-poor conditions (Ryther et al., 1981). Thalli of *Gracilaria* that has been exposed to elevated ammonium levels of 10 $mmolm^{-3}$ responded with an immediate increase in photosynthesis (Nelson, 1995).

G. lemaneiformis from the South China Sea have been grown well in bivalve culture areas in north China sea that after 116 days and the wet weight has been increased 89 times. In that study the amount of NO_2^- and NH_4^+ in water have been increased remarkably after the algae were harvested (Yang et al., 2005). The discharge of ammonia-rich effluent from fish and shrimp can result in the eutrophication of shore areas and this can be a valuable source for the commercial cultivation of seaweeds. The culture of *Gracilaria* is promising in this regard as thalli of this species are capable of rapid uptake and storage of nitrogen (Nelson et al., 2001).

Most other studies have concluded that ni trogen is generally the limiting nutrient for algae growth in marine environments (Hanisak, 1990).

A direct test of nitrogen versus phosphorous limitation has been conducted on *Gracilaria* and eight other fleshy seaweeds at Kaneohe Bay, using enrichment experiments and measurement of nutrients in sediments and the water column on the reef (Larned, 1998). Eight of the nine species (including *Gracilaria*) have been stimulated by ammonia, whereas phosphorous only stimulated one alga, *Codium edule*. The main finding of this project was that the biomass of *G. corticata* showed positive correlation with ammonium concentrations (P=0.022).

As in this study, the similar results have been obtained for *G. foliifera*, *Neoagardhiella bailey* (Deboer, 1979) and *G. gracilis* (Marinho-Soriano and Bourret, 2003). So, the highest phyco colloid content correlated with the greatest concentration of nitrogen. Similarly, cultivation of Neoagardhiella bailey over 45 days under continuous addition of nitrogen have shown higher yield of carrageanan (Deboer, 1979). But, agar from some red algae such as *Gelidium* sp. in Spain and *Gelidium pristoide* in South Africa (Freile-Pelegrin et al., 1995), *Gracilaria* sp. in Russia (Skriptsova et al., 2001) and carrageanan from *Chondrus* sp. (Deboer, 1979) have shown highest content at lower concentration of nitrogen.

In conclusion the highest amounts of biomass and agar yield coincided with high concentration of ammonium. It has a potential to use in high ammonia content waters. These findings on the effects of environmental factors on growth and agar of *G. corticata* could be utilized as an indicator of tolerance and optimal conditions for cultivation. Besides, the agar yield from alga cultivated at $0.08gl^{-1}$ ammonium phosphate was 22.97±1.81% is within the value range in industrial requirements (17-25%) that makes *G. corticata* as one of the commercial agarophytes.

Acknowledgments

The authors would like to thank Research Station of Mollusks of the Persian Gulf to support in sampling and experimental process, Campus of Science and Research, Islamic Azad University in agar extraction and all the people who have helped them.

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