Borneo Journal of Marine Science and Aquaculture
Volume: 05 (02) | Dec 2021, 53-56

Growth of *Gracilaria manilaensis* Yamamoto et Trono (Rhodophyta) under different light intensities, salinities and pH

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Abstract

Previous studies have demonstrated that the seaweed, *Gracilaria manilaensis*, has a high potential of producing agar for the food industry, and is a promising candidate as a dietary supplement for treating cancer and neurological disorders. Unfortunately, not many farms culturing *G. manilaensis* existed particularly in Malaysia. One of the reasons is limited knowledge of suitable environmental conditions needed for efficient production of the seaweed. Therefore, this study was carried out to identify the best growth conditions for *G. manilaensis* under different light intensities, salinities and pH. To achieve this objective, *Gracilaria* sp. was collected from a farm and identified based on morphological characteristics before being subjected to three environmental variables simultaneously: light intensities (100 and 1000 lux), salinities (15, 20, 25 and 30 psu) and pH (7.6, 7.8 and 8.0) simultaneously. Specific growth rates were determined for all the treatments. The results showed that *G. manilaensis* preferred high light intensity (1000 lux), with the growth rate of 1.69 ± 0.08 g/days at the salinity of 15 psu and pH 7.6. No significant differences were found between salinity and growth rate, indicating that *G. manilaensis* can tolerate a wide range of salinity. The knowledge gained from this study can be used as a guide to increasing the production of *G. manilaensis* in indoor farming systems. This will ensure sustainable research for *G. manilaensis* and production.

Keywords: Mariculture, *Gracilaria,* Rhodophyta, Seaweed, Growth rate.

Introduction

Seaweeds are well known for their biochemical composition and active metabolites such as agar, alginate, carrageenan, carotenoids, terpenoids, sterols, phycobilins and phycocyanin (Cornish and Garbary, 2010). Although the usage of seaweed in nutritional, medicinal, pharmaceutical, bioengineering industry and agronomic applications is the forefront of new millennium research (Dhargalkar and Pereira, 2005; Nagappan and Vairappan, 2014; Buschmann et al., 2017), archeological discovery shows that seaweed has been part of human diet as early as 14,000 years ago (Dillehay et al., 2008). Today, the usage of the marine biomass is widespread in animal feeding, fertilizer, biofuel production, and as a biofertilizers and soil conditioner. Seaweed farming is even seen as a way to ameliorate the impact of climate change (Giole et al., 2017). Seaweeds are also praised for their contribution to food security (Hebbale et al., 2017).

Although wild harvest of seaweed is still practiced by seaweed industries especially in Europe, Canada, and Latin American, the global seaweed production mainly relies on monoculture, integrated, and through offshore cultivation systems (Buschmann et al., 2017; Gioele et al., 2017). Asian countries particularly China and Indonesia are the largest seaweed producers with each producing approximately 10 million tonnes of seaweed annually, followed by the Phillippines and Korean with an annual production of over 1 million tonnes. Malaysia, the Democratic Republic of Korea, Japan and Zanzibar contribute approximately 100 000 tonnes production of seaweed biomass each (Buschmann et al., 2017). Despite the increasing production, the quantity is still not enough to cater to the global demand (Nayar and Bott, 2015).

Red seaweed (Rhodopyte) is an important resource for the food and industry in Asia. Over 300 species of red macroalgae have been identified but 160 species have been accepted taxonomically (De Almeida et al., 2011). Over 100 species of seaweeds are exploited for phycocolloid production and *Gracilaria* sp. is one of the ten species that is actively cultivated as the main source of income in the industry (Nayar and Bott, 2015; Gioele et al., 2017). In 2017, FAO recorded that the annual income from *Gracilaria* cultivation has amounted up to US 1 billion. China, Indonesia and Chile are the main countries that cultivate and export *Gracilaria* to cater to the world demand (Buschmann et al., 2017; Kim et al., 2017). This seaweed genus is attractive for commercial cultivation owing to its varieties of bioactive metabolites, its capacity in producing high yield and resilience to a wide range of environmental changes including salinity and temperature (Phooprong et al., 2007; de Almeida et al., 2011; Gioele et al., 2017).

In Malaysia, edible *G. manilaensis* is cultivated in small farms commonly together with other marine species such as mud crab and fish. The integrated farming was applied to increase the income of farmers as the seaweeds harvested were low in price and also to maintain the water quality for the healthy growth of the farm's fish, the main target. The dried seaweeds were sold at only RM3.50/kg whereas the mud crabs were sold at RM30/kg. Therefore, identifying an optimum environmental condition for the high growth of local *G. manilaensis* is needed. This will help farmers to increase seaweed production for better income generation. Furthermore, there are increasing reports that indicated the importance of species which contain antioxidant and cytotoxicity activities against cancer cell lines (Abdullah et al., 2013) and production potent bioactive compounds that mimic the neuroactivity of the nerve growth factor (Phang et al., 2018) besides being a source of agar (Ahmad et al., 2011).

Understanding the importace of this species, we hypothesized that *G. manilaensis* growth rate can be manipulated under different light intensities, pH, and salinity. In order to examine the correlation, cultivation under laboratory was conducted. The study also aims to develop a baseline information for optimum cultivation techniques that will lead to higher seaweed production.

Materials and Methods

Sample collection

Gracilaria sp. was collected from a fishpond at Pantai Merdeka, Kedah, Malaysia. The seaweed was washed and rinsed with seawater to remove dirt and placed in a tank filled with seawater and allowed to acclimatize for seven days as described in Nor-Salamah et al. (2015).

Identification of G. manilaensis

Morphology of *G. manilaensis* was compared with previous descriptions. A cross section of the thallus was made to observe the arrangement of cortex and medulla under the microscope.

Experimental culture of G. manilaensis

The experiments were set up following Nor-Salamah et al. (2015). A series of 3 × 3 × 2 factorial experiments consisting of two light intensities (100, and 1000 lux), three pH values (7.6, 7.8 and 8.0) and four salinities (15, 20, 25 and 30 psu) were conducted in triplicates simultaneously in the laboratory. For light intensity, a preliminary study was carried out in the laboratory to determine the range. For different salinities, freshwater or crystal salts were added to the seawater to gain the desired concentrations. About 4 g (fresh weight) of *G. manilaensis* was cultured in 500 mL beakers containing 300 mL of seawater and gently aerated to facilitate nutrient uptake. The experiments were conducted for three weeks (21 days) and the salinity and pH were checked every day to maintain the desired concentration.

Determination of specific growth rate (SGR)

Growth was determined weekly for 21 days. The specific growth rate was calculated based on Lobban and Harrison (1994) and was expressed as the percentage increase of weight per day.

$$SGR = [\ln (m_1/m_0)] / t*100$$

Where;

m_1 = biomass on day t
m_0 = initial biomass
t = time in days

Statistical analysis

Three-way ANOVA was performed with SPSS 16.0 program to determine the significance of differences of each of the parameters (light intensities, salinities and pH) with growth. Pearson correlation was used to determine the relationship between each parameter with the specific growth rate.

Results and Discussion

Taxonomic features of G. manilaensis Yamamoto et Trono

The morphology of *G. manilaensis* fits well with the previous descriptions (Yamamoto and Trono, 1994). Thallus were thin and dark red to brown in colour (Figure 1A). However, the species can also be found in purplish or green in colour. *Gracilaria manilaensis* collected was smaller in size with the length of main axis only up to 25 cm whereby the species measured up to 60 cm. Branching is irregular with cylindrical and compressed branches, constricted at the point of attachment. According to Yamamoto and Trono (1994), the extreme constriction at the branch bases is one of the distinctive features in identifying this species. According to them, *G. manilaensis* differed from *G. blodgetii* and *G. changii* in frond length, frond width and number of branches.

The cortex consisted of 2 layers and polygonal medulla (Figure 1B). The species was collected in brackish water, muddy bottom with the salinity of 16 psu, temperature of 32.6°C and pH of 7.68. The species can be found in sheltered environments of bays, estuaries and river mouth and was seen to grow in area with sandy or sandy-muddy bottom (Yamamoto and Trono, 1994; McHugh, 2003).

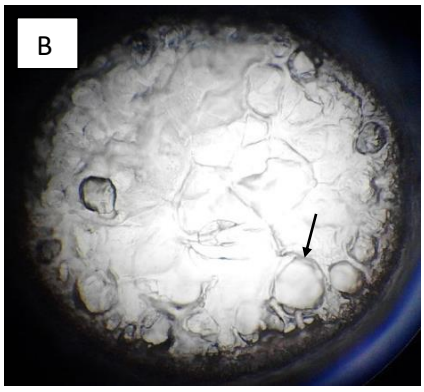

Figure 1. (A) *Gracilaria manilaensis* collected from the fishpond. (B) Cross section of *G. manilaensis* thalus showing polygonal medulla (arrow).

Growth rate of G. manilaensis

Abiotic factors including light, pH and salinity are important in determining the growth of seaweeds. Commonly, seaweeds do not easily to survive outside their natural environment due to the change in ambient environmental conditions (Agrawal, 2012). Therefore, research on the optimum condition for growth of *G. manilaensis* is important for developing an improved system. From this study, exposing *G. manilaensis* to different parameters simultaneously will somehow mimic its natural conditions. Commonly, growth studies in the past were conducted by using a single parameter.

It is evident from Figure 2 that the highest specific growth rate for 100 lux was 1.50 ± 0.02 g/days was recorded at 30 psu and pH 7.8 whereas the lowest growth rate was 0.34± 0.07 g/days noticed at the salinity of 25 psu and pH 8.0. For 1000 lux (Figure 3) the highest specific growth rate was 1.69 ± 0.08 g/days observed at 15 psu and pH 7.6. The lowest specific growth rate was 0.61 ± 0.08 g/days observed at 30 psu and pH 8.0 (Figure 3).

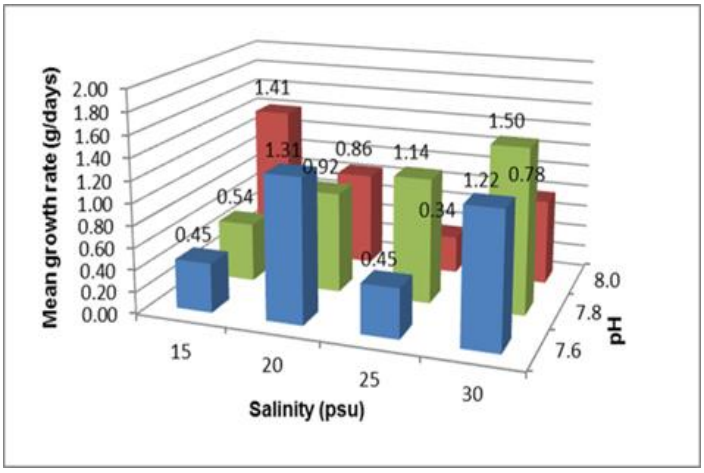

Figure 2. Growth rate of *Gracilaria manilaensis* at different pH and salinities at 100 lux.

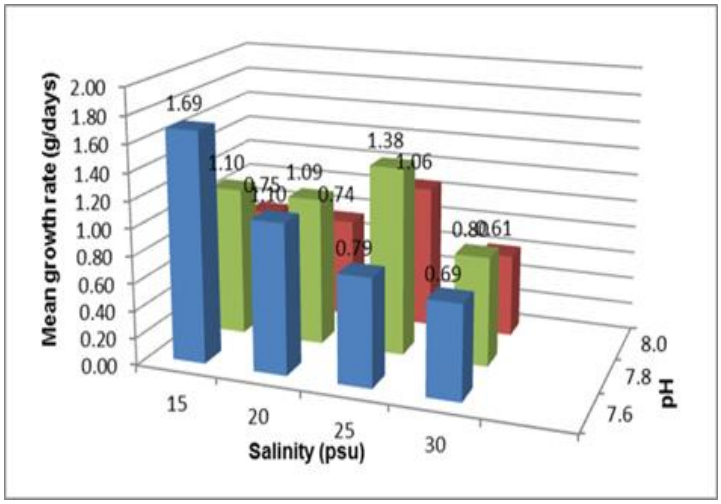

Figure 3 Growth rate of *Gracilaria manilaensis* at different pH and salinities at 1000 lux.

Based on statistical analysis, significant differences ($p<0.05$) related to light intensity and pH were observed. No significant difference ($p>0.05$) was found between salinity and specific growth rate. Positive correlation was recorded between specific growth rate and light intensity ($R^2 = 0.338$) but a negative correlation was found between specific growth rate and pH ($R^2 = -0.27$).

The results showed that different salinities did not affect the growth of *G. manilaensis* at different light intensities. This indicates that the species can tolerate a wide range of salinity from 15 to 30 psu. Most of *Gracilaria* spp. are reported to be euryhaline (Carton et al., 2011) whereby they can tolerate a wide range of salinity, with the optimum condition was between 25 to 35 psu (Raikar et al., 2001; Bunsom and Prathep, 2012). However, some species are able to grow better at a specific range of salinities. For example, *G. gracilis* and *G. edulis* preferred salinity of 10-15 psu (Skriptsova and Nabivailo, 2009; Yu et al., 2013).

Light intensity played a crucial role in *G. manilaensis* growth. Specific growth rate of *G. manilaensis* at 1000 lux was higher compared to that 100 lux whereby the highest growth recorded was 1.69 ± 0.08 g/days at salinity of 15 psu and pH 7.6 (Figure 3). The lowest specific growth rate was 0.34± 0.07 g/days recorded at the salinity of 25 psu and pH 8.0 (Figure 2). Previous studies reported that *Gracilaria* may have different adaptation depending on species. *Gracilaria lichenoides* and *G. cortica* grew best at 80 µmol photons m^{-2} s^{-1}(5920 lux). Light intensity is important in seaweed growth because it can influence the biochemical compounds of these macroalgae by increasing or decreasing their contents (Cirik et al., 2010; Sing and Singh, 2015). Results also showed that pH seems to affect seaweed growth more at slightly alkaline condition (7.6) compared to pH 8.0. Similar study on *G. manilaensis* at 500 lux also indicated that the species has better growth at pH 7.6 compared to pH 8.0 (Nor-Salamah, 2015).

Conclusion

Gracilaria manilaensis has potential to be cultivated due to its relatively high growth rate and tolerance to environmental condition. It also has a great potential for commercialization. This study has shown that the growth of *G. manilaensis* can be manipulated using different light intensities, salinity and pH. High light intensity (1000 lux), low salinity (15 psu) and pH of 7.6 were the best conditions to culture it for high growth rate and yield. This information is important for indoor propagation of the seaweed to ensure high production. Through a sustainable cultivation and continuous studies, more supplies can be assured to meet the market. demand.

Acknowledgement

This research was supported by Fundamental Research Grant Scheme (FRGS 14-134-0375). The authors wish to express their gratitude to the laboratory staff of INOCEM and students.

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