Chapter 28
Gracilaria Cultivation and the Role of Its Associated Bacteria for Biomass Production

V. R. Umashree and K. Arunkumar

28.1 Introduction

The importance of ocean as a source of protein is well known. Since the ocean covers ¾th of the earth surface and 2 to 3 times more productive than landmasses it gained much attraction recent years. Recently the fishing activity is increased largely all over the world (Raghu Prasad 1964). Together with this, the utilization of algae as a source of food and as a source of other secondary metabolites has also increased tremendously. Long-years back countries like China, Japan, and South Korea used algae as food. But now a days many other countries involved in the cultivation and utilization of algae when the researches showed their benefits to mankind.

One of the most important problems faced by our generation is global warming and climate change. Global warming is the term used to describe a gradual increase in the average temperature of the Earth's atmosphere and its oceans. According to the scientific consensus on climatic changes, the average temperature of the Earth has risen between 0.4 and 0.8 °C over the past 100 years (https://www.livescience.com/topics/global-warming). Research has shown that the carbon dioxide (CO_2) released into the atmosphere has increased significantly since the beginning of the industrial era (https://www.globalccsinstitute.com/institute). The increased volumes of carbon dioxide and other greenhouse gases released by the burning of fossil fuels, land clearing, agriculture, and other human activities, are believed to be the main sources of the global warming (https://www.livescience.com/topics/global-warming).

Carbon sequestration is the process of capturing atmospheric carbon and depositing it in reservoirs, a process to mitigate global warming and climate change

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(Dhanwantri et al. 2014). Various reports suggested that harvested seaweeds can be processed as biofuel feedstock which shall offer additional carbon sequestration potential (Zacharia et al. 2015; Mohammad et al. 2019; Hessami et al. 2019). They also suggested the conversion of the large seaweed biomass into biological charcoal known as biochar through pyrolysis which has agricultural applications (Zacharia et al. 2015).

Haoyang (2018) suggested that algae cultivation would reduce the greenhouse effect in the atmosphere. Algae can absorb carbon dioxide. Carbon, together with the remnants of algae, would be stored in deep oceans, on the seafloor, for several centuries. Since the algae have high metabolism rates and can also provide shellfish with abundant food that contains carbon. Shellfish's shells, which are difficult to be decomposed, are reliable storage of carbon, compared to dead organisms like trees and algae. They suggest that since the algae are fast-growing species, the effort we would need to spend to cultivate them is very little (Haoyang 2018).

Recently the bacteria associated with algae have gained much attraction due to their ability to produce a variety of secondary metabolites. Studies have also shown that these bacteria help in enhancing the growth of algae. Apart from these they also protect the algae from other pathogens and heavy metals.

Gracilaria is a group of warm water seaweeds with a great range of temperature and latitudinal tolerance. Some species were used as food (Shama et al. 2019) and as binding material in the preparation of lime for painting walls in China. On the discovery of agar content, its use is expanded to several Asiatic countries. From then diversity of cultivation methods have been developed in other different places (FAO 2014–2021).

With all these backgrounds, this review focuses on the different methods of cultivating the red macroalgae *Gracilaria* species, the significance of bacterial role in growth promotion and the importance of improving cultivation to gain multiple benefits including blue carbon sequestration.

28.2 *Gracilaria* Stock for Agar Production

According to the statistics reported by the Food and Agriculture Organization of the United Nations (FAO 2014–2021), there is about 2,257,919 tonnes of *Gracilaria* were reported by the year 2011. About 94.2 percent (some 697,240 tonnes) is produced by the cultivation while the remaining (42,224 tonnes) are gathered from wild stocks. The reports also indicate that the most productive countries in America are Chile, Peru and Argentina. In Asia, there is productive cultivation in Indonesia,Vietnam, the Philippines, and Korea while in Africa only Namibia reports *Gracilaria* production (FAO 2014–2021).

According to the FAO (2014), *Gracilaria* is one of the world's most cultivated seaweeds with over 0.8 million tons of annual production and nearly $160 million annual values (Kim et al. 2014). According to the Food and Agriculture Organization of the United Nations (2017), it is the world's most cultivated seaweeds with over 3.8 million tons of annual production and worth annually about US $1 billion (Kim et al. 2017). Most of the biomass of *Gracilaria* is used in the phycocolloid industry as the main source of food-grade agar (Pereira and Yarish 2008; Kim et al. 2017) and as an animal feed (Johnson et al. 2014; Kim et al. 2017; Shama et al. 2019). *Gracilaria* contributes about 66% of the total agar production (Pereira and Yarish 2008; Kim et al. 2017).

It is expected that the Asia Pacific is the largest producer of agar due to the easily available raw material – the red algal species in eastern and southwestern Asian countries. North America is expected to be the second-largest one. It is widely used in the food consumed by the North American population, which plays a crucial role in increasing the overall demand in the region (http://www.algaeindustrymagazine.com/new-report-explores-agar-market/).

Global agar production increased from 7500 to 9600 tonnes, with sale prices of USD 17/kg in 1999, increasing to USD 18/kg in 2009. The world agar sale value increased from USD 128 million in 1999 to USD173 million in 2009. In 1999, about 63 percent of the total agar production was produced by *Gracilaria* which have been increased to 80 percent in 2009 (FAO 2014–2021). Among the seaweed cultivation around the world, the genus *Gracilaria* ranked highest with over 3.8 million tons of annual production. It contributes about 91% of total agar production (Kavale et al. 2018).

In 2016, Agar for Food Industry occupied more than 57% of the total production of agar world wide. Since the agar is widely used in Food Industry, Pharmaceutic, Cosmetics, Daily Chemical, and Scientific Research, the demand for agar is expected to increase during the remaining years of the forecast period of 2017–2022. The global Agar market is valued at 280 million US$ in 2017. With a growth at a CAGR of 4.0% during 2018–2025, it is expected to reach 380 million US$ by the end of 2025 (http://www.abnewswire.com/pressreleases/global-agar-market-2018-industry-key-players-trends-sales-supply-demand-analysis-forecast-to-2025_234917.html).

There is a high pressure on wild populations of *Gracilaria* because of the high harvesting of wild *Gracilaria* crops and an increase in demand for agarophytes (Guanzon and de Castro 1992; Wilson and Critchley 1997). To reduce the over-exploitation of wild crops and to meet the ever-increasing demands for agarophytes and their products, there have been rapid developments in *Gracilaria* mariculture during recent years (Hansen et al. 1981; Wilson and Critchley 1997).

However, information about the influence of environmental parameters such as temperature, irradiance, salinity, and nutrients on the growth of the alga is required to evaluate commercial utilization of *Gracilaria* spp., (Rebello et al. 1996; Wilson and Critchley 1997).

28.3 Agar Yielding Potential Red Algal Species

The genus *Gracilaria* belongs to the class *Rhodophyceae* (Red algae). Most of them are marine species except a few ones. They are having well-developed branched thalli and multicelluar form. They vary in size and shape. They grow in crust on the rocks or shells as a large fleshy, branched, or blade like thalli. The thallus is basically filamentous. It may be simple or branched, free or compacted, and thus forming pseudoparenchyma with uni or multi axial construction (Fig. 28.1). They occupy intertidal to subtidal zones of coastal areas (Jones 1959; Kolanjinathan and Stella 2011).

Members of *Gracilaria* are among the most economically important seaweeds because of their ability to achieve high yields and to produce commercially valuable extracts (Cynthia et al. 2011). They are having a variety of uses, ranging from traditional foods and medicines to biological and industrial applications (Gressler et al. 2010; Kim et al. 2017). They are important for the industrial and biotechnological uses because of the presence of phycocolloids in their cellwalls mainly the agar (Cynthia et al. 2011).

The important and commonly occurring agar yielding seaweeds in different localities of Indian coast are species of *Gelidiella, Gracilaria, Gelidium and Pterocladia*. Among these red algae, only *Gelidiella acerosa, Gracilaria edulis,*

Fig. 28.1 *Gracilaria edulis* (S.G. Gmelin) P.C. Silva fresh thallus found along the coast of Tondi (9° 44′ 30“ N; 79° 1’ 3” E), South India

G. corticota var. corticata G. foliifera and G. verrucosa are available in exploitable quantities (Kaliaperumal and Kalimuthu 1997).

There are three quality grade agars are produced namely, sugar reactive agar, standard agar and food-grade agar. In the sugar reactive agar, the gels are stronger as a function of sugar concentration. It is obtained largely from *Gracilariopsis lemaneiformis*, the most important species under cultivation in China at present (FAO 2014–2021). In the standard agar the gel has the temperature, consistency and structure for microbiological purposes. It is produced largely by *Gelidium, Pterocladia* or *Pterocladiella*. Any kinds of agar that are not meeting the requirements for sugar-reactive or bacteriological agar are designated as the food-grade agar. It is extracted from a wide variety of *Gracilaria* species (FAO 2014–2021).

28.4 Methods of Cultivation

Gracilaria species are mainly cultivated using vegetative fragments. For its cultivation, sustainable seedstock is very important. Seedstock has been supplied from the wild; either the healthy branches of *Gracilaria* from natural stock were collected or reproductive plants were selected to collect spores (either carpospores or tetraspores) for seeding (Buschmann et al. 2008; Kim et al. 2017).

The tropical species of agarophytic seaweeds can be obtained either from gathering natural stocks which is greatly influenced by seasonal changes in the weather (monsoons) or from farming or culturing of these species which is more predictable and stable, and targeted outputs are easily attained (Gavino 1989).

According to some reports,*Gracilaria* cultivation is done mainly in three different ways, including open water, pond, or tank cultures (Pereira and Yarish 2008; Kim et al. 2014). But some other reports say that it is being cultivated in four ways including open water rope cultivation, nearshore bottom cultivation, pond culture, and tank cultures (Kim et al. 2017). Though there are many more methods are practiced for the cultivation.

Open water cultivation is practiced in estuaries, bays, and upwelling areas. A nursery (tank culture) system provides sufficient seedstock (Pereira and Yarish 2008).

In the **pond culture** of *Gracilaria*, the water is introduced into the pond that is dried for several days. Seaweed cuttings are directly staked onto the bottom (Castanos and Buendia 1998).The non-intensive ponds are usually made of an uncovered earthen construction and are lack an artificial water agitation system, while the intensive cultivation ponds are made of a concrete or plastic structure with a water agitation system (Friedlander and Levy 1995).

Tank cultivation has the advantage in its simplicity of controlling the culture system (Pereira et al. 2013). This ensures that production meets high quality standards and biosafety for human consumptions, as well as for other high-value applications such as cosmetics or pharmaceutical products.

In the **rope cultivation** either vegetative materials are tied or inserted within a rope or spores are left to settle on the surface of the ropes. The ropes or lines used here can be monofilament, nylon or other suitable lines. Suspended rope culture is a relatively simple fixed grow-out system (Yarish et al. 2012). Seeded ropes are suspended, stretched between stakes buried in the sediment, or supported at different levels by buoys or rafts (FAO 2014–2021).

In **near shore bottom cultivation,** fronds are either 'seeded' onto rocks and spread on the bottom in a shallow area, or attached to lines that are strung on stakes and suspended just above the bottom (Chen 1989). Here the spore collection and farm sites are usually the same (Veeragurunathan et al. 2015).

In the **floating culture** method, seaweed stocks are inserted in seedling rope made up of palm thread or artificial fiber which isthen fixed to a floating raft (Chen 1989).

In **tube- net method**, tube-net modules (polypropylene commercial fishnet) are used (Pereira and Yarish 2008). After seeding directly lengthwise into the tube they are tied to floating bamboo raft (Ganesan et al. 2017; Mantri et al. 2017).

In the **net bag method,** about 200–300-g seedlings are seeded onto a 75-cm-long bag prepared from commercial fishnet which is covered with agro net. Then the bag is tied onto an 8-mm polypropylene rope. The rope is then tied on both sides to the vertically erected bamboo poles (Ganesan et al. 2017).

In **net pouch method**, net pouch is made with a 3-m-long tube. The bag is then made in to five equal compartments by a hand stitch up to the nylon thread in which *Gracilaria* fragments are seeded (Ganesan et al. 2017).

28.5 Bacterial Association in the Algal Growth

Microorganisms are found everywhere, in all ecosystems around the globe. They can survive even in extreme conditions. It is suggested that at least ten million microbial species remaining unidentified in nature. Only less than 1% of all bacterial species and less than 5% of all fungi species are described (Berdy 2012). Goecke and his colleagues mentioned that seawater containsup to 107 viruses, 106 bacteria, 103 fungi per ml (Goecke et al. 2010). Macroalgae are highly susceptible to epibiosis. Because of the rich content of organic material, a number of bacterial species get associated with macroalgae (Goecke et al. 2010).

Previous studies reported that the number of seaweed-associated bacteria is 100–10,000 times more than those from the surrounding seawater (Chan and McManus 1969; Weinberger et al. 1994). Sutha et al. (2011) enumerated 15 bacterial isolates from six seaweeds (*Gracilaria edulis, Hypnea valentiae, Acanthophora spicifera, Enteromorpha intestinalis*, *E. flexuosa, Ulva lactuca*) and identified the presence of *Bacillus licheniformis*, *B. subtilis*, *B. pumilus*, *B.marinus*, *Staphylococcus aureus* and *Streptomyces coelicolar* (Sutha et al. 2011).

Seaweed associated bacteria so far isolated belong to the (super) phyla Proteobacteria, Actinobacteria, Bacteroidetes (CFB group), Cyanobacteria, Firmicutes, Planctomycetes, Verrucomicrobia, Chloroflexi, Deinococcus-Thermus, Fusobacteria, Tenericutes, and the candidate division OP11 (Hollants et al. 2012).

In the review of Hollants and collegues (2012), they mentioned that, in all studies reviewed, the most common bacterial clade associated with seaweeds were Gammaproteobacteria with 37% relative abundance (percentage of published records), followed by the CFB group (20%), Alphaproteobacteria (13%), Firmicutes (10%), and Actinobacteria (9%). On a lower taxonomic level, the orders Flavobacteriales (14%), Alteromonadales (12%), Vibrionales (10%), Pseudomonadales (9%), Bacillales (9%), Actinomycetales (8%), and Rhodobacterales (7%) were most abundant in seaweed-associated bacterial communities (Hollants et al. 2012).

Suvega and Arunkumar (2014), isolated 673 bacteria from different algae *Caulerpa scalpelliformis*, *Ulva lactuca*, *U. fasciata, Chaetomorpha linum, Gracilaria edulis*, *G.corticata* var. *corticata*, *Hypnea valentiae*, *Grateloupia filicina, Kappaphycus alvarezii and Sargassum wightii* as well as from sediments and seawater. They reported the presence of 26 bacterial genera with with species of *Bacillus* recording a maximum of 40.2%. They indicated that bacterial populations were considerably higher in seaweeds as compared to seawater and sediments (Suvega and Arunkumar 2014).

Algal–Bacterial Interactions Marine algae and bacteria have come a long way since algal plastids originated from Endosymbiotic Cyanobacteria (Margulis and Schwartz 1998; Ramanan et al. 2016). They developed a diversified bacterial association like beneficial (mutualistic), harmful (parasitic), neutral (commensal), etc. Bacteria either live on the surface (epiphytes) or in the cytoplasm and/or vacuolar systems of the cells (endophytes) (Hollants et al. 2012; Friedrich 2012).

Beneficial Bacterial–Macroalgal Interactions Seaweed-associated bacteria gained a lot of importance because of theirability to produce a variety of secondary metabolites. A large number of endophytic, epiphytic and epibiotic bacteria are associated with macroalgae, which constantly interact with their host positively or negatively.

Studies by Sturz et al. (2000) have shown that bacterial isolates can contribute to the growth, health, and development of seaweeds by the direct production of the plant growth regulators and nitrogen fixation (Sturz et al. 2000). These plant growth regulators have been reported to play an important role in the morphogenesis of callus from Rhodophyta members (Yokoya 2000; Reddy et al. 2003).

Bacterial effects on morphogenesis have been reported in foliaceous green macroalgae such as *Ulva* and *Monostroma*. It has been reported that this morphogenesis controlled by bacteria belonging to the genera *Cytophaga*, *Pseudomonas*, *Staphylococcus*, *Vibrio*, *Bacillus*, and *Flavobacterium* (Marshall et al. 2006; Singh et al. 2011a).

The plant growth-promoting nature of bacteria associated with *Laminaria japonica* was reported by Dimitrieva et al. (2006). In addition to these, studies showed that microorganisms play a role in the protection of macroalga against toxic heavy metals (Goecke et al. 2010) or crude oil (Goecke et al. 2010). Microorganisms are able to detoxify, for example, heavy metals by precipitation, adsorption, or transformation to fewer toxic forms (Yurkov and Beatty 1998; Goecke et al. 2010).

Bacteria produce morphogenic factors, fixed nitrogen, enzymes, and vitamins which promote algal growth. Morphogenesis is controlled by a highly potent differentiation inducer, thallusin, isolated from well-defined associated bacteria (Hollants et al. 2012).

Seaweed-associated bacteria were isolated and studied for their morphogenesis capability in axenic cultures of the green alga *Ulva fasciata* (Singh et al. 2011a). This was the first study providing evidence of the effect of bacterial isolates on zoospore induction. Later the role of bacterial isolates in enhancing the bud induction in the industrially important red alga *Gracilaria dura* was studied. The findings revealed for the first time that IAA coupled with nitrogen fixation induces and regenerates new buds in *G. Dura* (Singh et al. 2011b). They suggested that bud-inducing bacterial isolates are important for nitrogen supply to the *G. dura*. These associated nitrogen-fixing bacteria will fix atmospheric nitrogen and supply nitrogen supplement sources.

Studies by Weinberger et al. (2007) on *Acrochaetium* sp., showed that the spore release is controlled by bacteria. He reported that suppression of bacterial epiphytes with antibiotics prevented spore release of *Acrochaetium* sp. completely, that demonstrated that this alga strongly depends on bacterial AHL. They suggested that the life cycle completion in *Acrochaetium* sp. strongly depends on bacteria (Weinberger et al. 2007).

The studies done by Shin (2008) showed that enhanced settlement of spores on mixed microbial biofilm of *Ulva fasciata* (Shin 2008). The studies done by Imchen (2012) on the influence of biofilms on the zoospore settlement of *Enteromorpha flexousa* showed that the biofilms provide a favorable substratum for settlement (Imchen 2012).

Studies to evaluate the effect of quorum sensing (QS) molecules produced by the epiphytic and endophytic bacteria associated with green macroalgae *Ulva* (*U.fasciata* and *U.lactuca*) and red macroalgae *Gracilaria* (*G.corticata* and *G.dura*) on the carpospores liberation from *G.dura* has shown that carpospore liberation is positively induced by the isolated bacteria (Singh et al. 2015). Studies also demonstrated that some Gram-negative epiphytic and endophytic seaweed-associated bacteria produce different types of AHLs. They reported that these bacterial isolates can effectively be used for mass carpospore liberation.

These studies show the significance of associated bacteria in the growth of macro algae. It is obvious that there are bacteria that contribute to bud induction, spore release, spore settlement, morphogenesis, nitrogen fixation, etc. Thus, isolation and identification of such bacteria and their mechanism of interaction will be very helpful for improving the cultural practices of *Gracilaria* and other algae.

28.6 Potential of *Gracilaria* Mariculture in India

Within the red macroalgae, the genus *Gracilaria*, represented by more than 100 species (Oliveira and Plastino 1994) living in warm water areas (Steentoft and Farham 1997), is notable for its economic importance as a source of agar. Culturing of seaweeds in Indian coastal waters started with cultivation of *Gracilaria edulis* due to its high regenerative capacity (Zacharia et al. 2015). Currently, 185 *Gracilaria* species are taxonomically accepted (Guiry and Guiry 2016).

The surveys carried out by CSMCRI, CMFRI and other research organizations have revealed vast seaweed resources along the coastal belts of South India. On the West Coast, especially in the state of Gujarat, abundant seaweed resources are present on the intertidal and sub tidal regions (Govt. of Gujarath 2017). These resources have great potential for the development of seaweed-based industries in India.

There are about 25 agar and alginate industries situated in states of Tamil Nadu, Karnataka, Kerala Pondicherry, Andhra Pradesh and Gujarat. Among these, more than 20 industries produce food grade agar by using *Gracilaria edulis* (Kaliaperumal et al. 2004; Kaliaperumal and Ramalingam 2005).

Thirty-one species of *Gracilaria* are found on the Indian coastline of the Bay of Bengal. The estimated biomass of *Gracilaria* from Indian waters is 1700 tonnes (Krishnamurthy 1989). India produces 110–132 tons of dry agar annually utilizing about 880–1100 tons of dry agarophytes (Govt. of Gujarath 2017).

Central Salt and Marine Chemical Research Institute (CSMCRI), Marine Algal Research Station (MARS), Mandapam, Tamil Nadu a CSIR Institute developed viable and commercially sustainable methods for cultivating *Gracilaria edulis*. CSMCRI developed single Rope Floating Raft (SRFR) method which is suitable for culturing seaweeds in wide area and greater depth (Kaliaperumal and Kalimuthu 1997; Gulshad 2016).

Floating raft technology has been recommended to be used on the coasts of Kerala for agarophytic algal cultivation. Certain areas in the Gulf of Kutch have been suggested as suitable for deep-water seaweed cultivation. CMFRI has developed techniques for culturing *Gelidiella acerosa*, *Gracilaria edulis, Hypnea musciformis* and *Acanthophora spicifera*, and to find improved techniques for propagation and large-scale culture of other economically important seaweeds, attempts are being made (Gulshad 2016; Govt. of Gujarat 2017).

In different localities of Indian coast, *Gracilaria edulis, G. corticola var. corticata, G. foliifera* and *G.verrucosa* are available in exploitable abundant quantities. *G.arcuala* and *G. verrucosa* occur in harvestable quantities in some estuaries and backwaters of Tamil Nadu and Pondicherry (Kaliaperumal and Kalimuthu 1997). A 17-fold increase in yield of *G. edulis* was obtained in 76 days in the first harvest at Minicoy Lagoon, Minicoy Island, of Lakshadweep, India during south west monsoon season by adopting single bottom coir rope method (Krishnamurthy 1989; Kaliaperumal and Kalimuthu 1997).

Single bottom coir rope method and single bottom nylon rope method are best method for culture during the southwest monsoon season at Islands of U.T. of

Lakshadweep. This is cost effective seaweed farming technology and practiced by fisherman of Lakshadweep to generate income during lean fishing season of southwest monsoon (May to September) because this time site becomes enriched with nutrients, which help to get good growth of seaweeds (Kaliaperumal and Kalimuthu 1997; Gulshad 2016).

According to the project done by Government of Gujarat (2017), India can grow more than one million tonnes of seaweeds in six states-Gujarat, Tamil Nadu, Kerala, Andhra Pradesh, Maharashtra and Andaman & Nicobar Islands. In the global markets each tons of average quality agar-agar is sold for more than US$2000 (INR120,000) and the country has the potential to generate more than INR200 crore in foreign exchange annually apart from providing additional income and gainful employment to thousands of people on the coastline (Govt. of Gujarat 2017; Krishnamurthy 1989). This shows the importance of *Gracilaria* culturing in India.

28.7 Conclusions and Future Perspectives

The increasing population pressure and energy needs have resulted in issues related to global warming. To avoid the dangerous after-effects of this, there is need for ecofriendly solution. It also demands multi-pronged approaches. One of them being seaweed farming to achieve sustainable climate resilient strategy with multiple benefits as means of carbon sequestration and mitigation of ocean acidification, also as feedstock for bioenergy production with an underpinning of economic gains for the dependent populations. The red macro algae *Gracilaria* are economically very important seaweeds for their agar yielding properties and other biotechnological and industrial applications. A number of cultivation techniques are being developed for the *Gracilaria* as a sustainable alternative to natural stock which is already over expoloited. Knowledge on the life cycle and environmental factors that affect growth of *Gracilaria* will help to improve the techniques for cultivation. One of the ways to improve the algal growth is isolating and identifying growth promoting seaweed associated bacteria. The recent studies showed that the seaweed associated bacteria help in improving the growth of algae thus improving the biomass productivity. When compared to other countries, India is still improving methodologies for cultivation of *Gracilaria*. However, India, which has a large coastline area, has a high possibility for developing cultivation techniques and improving algae-based industries. It will offer jobs for millions of people and provide a large income to the nation. It will also address the issues of global warming and facilitate a move towards carbon-neutral economy.

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