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SEAWEED BIOTECHNOLOGY

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Introduction

Seaweeds or marine macro algae are primitive non-flowering plants without true root, stem and leaves. They form one of the commercially important marine living renewable resources. They are the only source for the production of phytochemicals such as agar, carrageenan and algin. Seaweeds occur in the intertidal, shallow and deep waters of the sea upto 180m depth and also in estuaries and backwaters. They grow on rocks, dead corals, stones, pebbles, solid substrata and on other plants. Based on the type of pigments, external and internal structures, seaweeds are divided into green, brown, red and blue-green algae. Seaweeds contain many trace elements, minerals, protein, iodine, bromine, vitamins and many bioactive substances.

The luxuriant growth of seaweeds is found in southeast of Tamil Nadu, Gujarat Coast, Lakshadweep and Andaman-Nicobar Islands. Rich seaweed beds occur at Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhinjam, Pulicat Lake and Chilka Lake. About 220 genera and 740 species of marine algae have been reported so far from Indian waters. The total standing crop of seaweeds from intertidal and shallow waters of all maritime states and Lakshadweep was estimated as 91,333 tonnes (wet wt.). The quantity of seaweeds estimated in deep waters of Tamil Nadu was 75,373 tonnes (wet wt.) in an area of 1863 sq. km. from Rameswaram (Dhanushkodi) to Kanyakumari (Kaliaperumal, *et al.*, 1987a; Kaliaperumal, 1994).

Seaweeds are utilised for the production of phytochemicals such as agar, carrageenan and alginate which are widely used as gelling, stabilising and thickening agents in many industries such as food, confectionary, pharmaceutical, dairy, textile, paper, paint and varnish, etc. Agar is manufactured from red algae like *Gelidiella, Gracilaria, Gelidium* and *Pterocladia*. Some other red algae *viz. Eucheuma, Chondrus, Hypnea* and *Gigartina* are used for the production of carrageenan. Alginate is obtained from brown algae such as *Sargassum, Turbinaria, Laminaria, Undaria, Macrocystis* and *Ascophyllum.* Other chemical products namely mannitol, iodine, laminarin and furcellarin are also obtained from seaweeds. Many protein rich seaweeds such as *Ulva, Enteromorpha, Caulerpa, Codium* and *Monostroma* (green algae); *Sargassum, Hydroclathrus, Laminaria, Undaria, Macrocystis* (brown algae) *Porphyra, Gracilaria, Eucheuma, Laurencia* and *Acanthophora* (red algae) are used as human food in the form of soup, salad, vegetable and porridge. The food products like jelly, jam, chocholate, pickle and wafer can also be manufactured from certain marine algae. Seaweeds are also utilised as animal feed and fertiliser for various land crops. Many bioactive compounds can also be obtained from seaweeds (Chennubhotla *et al.,* 1981, 1987a, 1987b; Kaliaperumal *et al.,* 1987b, 1995).

NEED AND ADVANTAGES OF SEAWEED CULTURE

In general seaweeds are cultivated for supply of raw materials to the seaweed industries and for their use as human food. There are several advantages in the cultivation of seaweeds. In addition to a continuous supply of alga, crop of single species could be maintained continuously. By adopting scientific breeding and other modern techniques of crop improvement, the yield and quality of seaweeds could also be improved. Further, if seaweed culture is carried out on large scale, natural seaweed beds could be conserved purely for obtaining seed materials (Krishnamurthy, 1967).

METHODS OF SEAWEED CULTIVATION

There are two methods for cultivation of seaweeds - one by means of vegetative propagation method using fragments from mother plants and the other by reproductive method using different kinds of spores such as swarmers, zoospores, monospores, tetraspores and carpospores. In the vegetative propagation method, the fragments are inserted in the twists of ropes, tied to nylon twine or polypropylene straw and cultured in the nearshore areas of the sea. The fragments are also cultured by broadcasting them in outdoor ponds and on shore tanks. The fragment culture method is a simple one and gives quick results. In the culture of seaweeds from spores, the spores are first collected on nets, bamboo splints, polypropylene straw and other suitable substrata, reared to germlings or young plants in the hatchery / nursery and then transplanted to the desired culture sites where they grow to harvestable size plants. In this method the spores take more period for their development to harvestable size plants when compared with the growth of fragments in the vegetative methods. The biotechnology applied in the culture of seaweeds in these two methods are given below.

Vegetative propagation method

The following techniques are adopted for the cultivation of various economically important seaweeds in different countries by vegetative propagation method (Chennubhotla *et al.,* 1987c; Kaliaperumal, 1993; Anonymous, 1996).

Fixed off bottom culture

In this culture technique, seaweeds are cultured above the sea bottom at fixed levels. The carrageen yielding algae *Eucheuma denticulatum* and *Kappaphycus alvarezii* are cultured on long line monofilaments. In this "tie-tie" method the seedlings are tied at one end of polypropylene straw (plastic or rafia) and the other end is tied to the stretched out nylon monofilaments, in the sea (Parker, 1974). *Gracilaria* spp. in Chile, Caribbean, Indonesia and Myanmar (Anonymous, 1996) and *Gracilaria edulis, Gelidiella acerosa, Hypnea musciformis, H. valemtiae, Acanthophora spicifera* and *Saragassum* spp. in India (Thivy, 1964; Raju and Thomas, 1971a; Umamaheswara Rao, 1974; Subbaramaiah *et al.,* 1975; Krishnamurthy *et al.,* 1975, 1977; Chennubhotla *et al.,* 1978; Rama Rao *et al.,* 1987; Rama Rao

and Subbaramaiah, 1986; Kaliaperumal *et al.,* 1986, 1992, 1996) are cultured on stretched lines or nets made of coir or synthetic fibre. The seedlings are inserted directly in the twists of the ropes in this method.

Floating raft/cage culture

In this method seaweeds are cultured at sub-surface level of the sea using rafts and cages. Raft culture method is followed for culture of *Gracilaria* spp. in Caribbean, *Gracilaria asiatica, G. lemaneiformis, G. verrucosa* and *G. sjoestedtii* in China and *Gracilaria gracilis* and *Gracilariopsis tenuiformis* in Namibia. Cage culture method is adopted for culture of *Gracilaria heteroclada* along with fish in Philippines and *Gracilaria verrucosa, Pterocladia capillacea, Hypnea musciformis* and *H. cornuta* in Isreal (Li Ren-zhi *et al.,* 1984; Ren Guo-Zhong *et al.,* 1984; Dawes, 1995; Anonymous, 1996).

Bottom culture

In this culture technique seaweeds are cultured at the bottom of sea, ponds, canals and onshore tanks. In Chile *Gracilaria* seed material are planted directly at sea bottom sand in the intertidal region or with the help of sand filled tubes in the subtidal region (Anonymous, 1996). In U.S.A. fragments of *Gracilaria verrucosa* are sowed in net pens in the sea. In India *Gelidiella acerosa* is cultured by wounding round the seeded nylon twines to the nails fixed on coral stones in the sea (Patel *et al.,* 1986).

Caulerpa lentillifera and *Gracilaria heteroclada* are cultured along with the prawn *Penaeus monodon* in the ponds using net cages at Philippines. In Taiwan *Gracilaria edulis, G. verrucosa* and *G. gigas* are cultured in ponds under polyculture system with the prawn *Penaeus monodon,* Milkfish *Chanos chanos* and mudcrab *Scylla serrata. Gracilaria* spp. in China and Vietnam and *Gracilaria verrucosa, Pterocladia capillacea, Hypnea musciformis* and *H. cornuata* in Israel are also cultivated in the ponds (Anonymous, 1996).

In Philippines *Gracilaria heteroclada* in canals and in Thailand *Gracilaria crata, G. verrucosa* and *Caulerpa lentillifera* in the waste water ponds and recycling ponds at shrimp farms are cultured (Anonymous, 1996).

Gracilaria tikvahiae, G. verrucosa, Ghondrus crispus, Hypnea musciformis and *Ulva lactuca* in U.S.A., *Gracilaria conferta* in Isreal and *Gracilaria changii* and *G. tenuspitata* with *Penaeus monodon* in Thailand are cultured in onshore brick tans, fibre-glass tanks and plywood tanks with running seawater system and providing aeration and nutrient media (Shacklock *et al.,* 1975; Lepointe and Ryther, 1978; Bidwell *et al.,* 1985; Friedlander and Levy, 1995).

Green house culture

In Canada *Chondrus crispus* and *Gracilaria* are cultured in the green house by manipulation of temperature and light (Neish and Fox, 1971; Edelstein *et al.,* 1976). In India also culture of different commercially important seaweeds such as *Gracilaria edulis, G. foliifera, G. crassa, G. corticata* var. *corticata, Hypnea valentiae, Acanthophora spicifera* and *Laurencia papillosa* have been attempted in green house condition with running seawater system and providing aeration.

Spray culture

The main advantage of spray culture system is that the cultured seaweeds are free from epiphytes. Dripping, spraying or misting applications are employed on land based seaweed farms in order to wet the seaweeds and to maintain a film of seawater on them. The spray, mist of drip system has some significant advantages over culturing algae under water in pools, lagoons or the sea. The most practical reason is that less water is required in this culture system.

By this spray culture technique *Gracilaria chilensis* was attempted using seawater enriched with sodium nitrate in outdoor. The following species were cultured using artificial seawater (modified Provasoli medium) - *Ascophyllum nodosum, Fucus serratus, F. vesiculosus, Halydris siliquosa, Laminaria saccharina* (brown algae), *Gracilaria, Furcellaria lumbricalis, Chondrus crispus, Polyides rotundus, Rhodomela confervoides* and *Polysiphonia* sp. (red algae). The following algae are cultured in running seawater on net trays in tiers - *Ulva lactuca* (green alga), *Fucus vesiculosus, Ascophyllum nodosum* (brown algae), *Furcellaria lumbricalis, Chondrus crispus, Ahenfeltia plicata, Gracilaria secundata, Pterocladia pinnata* and *Gelidium* sp. (red algae) (Moellar *et al.*, 1984; Lignell and Pedersen, 1986; Haglund and Pedersen, 1988; Pickering *et al.*, 1995).

Raceways culture

There are two advantages in the culture of seaweeds in raceways (land based culture system). Seaweeds can be grown in any location without the limitations imposed by natural shoreline locations and production can be continued even during the rainy season by protecting the raceways with translucent covers to prevent dilution of seawater by rains. The fast growing tropical strain *Gracilaria N Br-10* is cultivated in raceways (ponds with water current) at Philippines. It has a high agar content of more than 18% and gel strength of 680 - 950 g/cm^2.

Tissue culture

A high yielding and fast growing strain of seaweeds with improved quality of phycocolloids can be produced by tissue culture technique. The other advantages of tissue culture are (1) Disease free varieties, (2) Hybrid strains for better quality and higher yields of agar and alginates, (3) Extraction of phycocolloids directly from the callus tissues, (4) Introduction of new species like *Eucheuma* and *Chondrus crispus* in the Indian waters.

Cell culture and protoplasmic fusion (somatic hybridization) are the most advanced and efficient methods for the production of hybrid strains with desired traits. They are the quickest and easiest method to develop such strains of desired characters *i.e.*, selection of hybrid strains for high colloid content, fast growth, high biomass production and improved quality of colloids. The protoplast isolation technique was used in red algae by most of the workers.

In foreign countries culture of the following algae were attempted by tissue culture technique *Ulva linza, Enteromorpha linza, E. intestinalis, Ulvaria oxysperma, Codium, Acetabularia, Monostroma angicera* (green algae); *Undaria pinnatifida, Ecklonia radiata, E. cara, Laminaria digita, L. hyperborea* (brown algae); *Porphyra linearis, P. suborbiculata, P. yezoensis, Porphyra* spp., *Gelidium versicolor,*

Grateloupia doryphora and *Laurencia* sp. (red algae). In India, culture of *Porphyra vietnamensis, Hypnea cervicornis* and *Sargassum tenerrimum* was carried out by application of tissue culture (Deshmuke, 1989). Some Indian species like *Gelidiella acerosa* which is endemic to certain areas, is one of the best raw materials for agar industry. This species can be grown throughout the Indian coast using tissue culture techniques.

Reproductive propagation method

In this method healthy reproductive plants collected from the wild are transported to the laboratory / nursery and different types of spores such as swarmers, zoospores, tetraspores, carpospores and monospores are collected on various substrata such as nylon twine, nylon rope, synthetic rope, coir rope, plastic strip (polypropylene straw / rafia), bamboo splint ladder, cement blocks, coral stones, etc. The spores on the substrata are cultured into sporelings in the culture room / hatchery by manipulation of temperature, light and providing nutrient culture media. Then the substrates containing sporelings / germlings are transferred to the suitable culture areas in the sea for their further growth to harvestable size plants. This method is followed for the commercial scale cultivation of edible red alga *Porphyra* and green algae *Enteromorpha* and *Monostroma;* agar yielding red alga, *Gracilaria cylindrica* and algin yielding brown algae, *Laminaria, Undaria* and *Macrocystis* in foreign countries such as Japan, China, Korea, Taiwan, Malaysia and U.S.A. (Chennubhotla *et al.*, 1987c; Santelices and Doty, 1980; Kaliaperumal, 1993).

In India experimental culture of the agar yielding red alga, *Gracilaria edulis,* algin yielding brown alga, *Sargassum plagiophyllum* and edible green algae, *Enteromorpha flexuosa* and *Ulva fasciata* were carried out successfully by spores method (Krishnamurthy *et al.*, 1969; Raju and Venugopal, 1971b; Ohno *et al.*, 1981; Oza *et al.*, 1985a, 1985b; Mairh *et al.*, 1986; Reeta Jayasankar and Kaliaperumal, 1991).

PROSPECTS IN APPLICATION OF BIOTECHNOLOGY IN SEAWEED CULTURE FOR COMMERCIAL SCALE PRODUCTION IN INDIA

In India at present seaweeds collected from wild are used as raw material for the production of agar and alginates. Nearly 25 agar and 10 algin industries are functioning at different places in the maritime states - Tamil Nadu, Kerala and Karnataka. About 5000 tonnes (dry wt.) of alginophytes *Sargassum* spp., *Turbinaria* spp. and *Cystoseira trinodis* and 500 tonnes (dry wt.) of agarophytes *Gelidiella acerosa, Gracilaria edulis, G. crassa* and *G. foliifera* exploited from the natural seaweed beds mostly from south Tamil Nadu coast are used as raw materials by these industries (Kaliaperumal and Kalimuthu, 1997). These quantities, particularly agar yielding seaweeds, are inadequate to meet the raw material requirements of Indian seaweed industries. Many seaweed industries are coming up every year. There is an increasing demand for the raw materials, which the existing resources cannot meet (Kalimuthu and Kaliaperumal, 1996). Hence, commercial scale cultivation of seaweeds is necessary for uninterrupted supply of raw material to the industries.

So far, only experimental scale cultivation of commercially important seaweeds such as *Gelidiella acerosa, Gracilaria edulis, Hypnea musciformis, Acanthophora spicifera* and

Sargassum spp. at different field environments using various culture techniques of vegetative propagation method and *Sargassum plagiophyllum*, *Enteromorpha flexuosa*, *Ulva fasciata* and *Gracilaria edulis* by reproductive method using spores were carried out successfully in our country. The various biotechnological aspects being applied in large scale cultivation of *Porphyra* (Japan, Korea, Taiwan), *Undaria* (Japan, Korea), *Laminaria* (China, Japan) by reproductive porpagation method and *Eucheuma* and *Kappaphycus* (Philippines), *Gracilaria* (Taiwan), *Hypnea* (Philippines), *Chondurs* and *gigartina* (Florida) and *Caulerpa* (Philippines) by vegetative propagation method can be followed for the production of commercially important seaweeds on large scale to meet the raw material needs of the seaweed industries in our country and to conserve the natural seaweed resources of Indian waters for using as seed material for commercial scale cultivation.

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