CHAPTER 1

Introduction

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1.1 Importance of Seaweeds

Seaweeds are widely distributed in all oceans of the world but their importance has been underestimated. Marine algae are important sources of food, supplements in animal feeds and pharmaceuticals. They have also been used as fertilisers and soil conditioner among others. Seaweeds, being important dietary supplement, at least to the Japanese, is best illustrated by the 'Nori' (*Porphyra*) industry in Japan which alone turns over more than one billion dollars per annum (McLachlan, 1985). Many seaweeds have been recognised as potential sources of bioactive natural products ranging from sterols, terpenoids to brominated phenolics that show bioactivity against microorganisms (Wong *et al.*, 1994).

The major groups of seaweeds include Chlorophyta (green algae), Pheophyta (brown algae) and Rhodophyta (red algae) although the most important industrial products are phycocolloids from Phaeophyta and Rhodophyta. Phycocolloids are polysaccharide complexes classified into three generic types: alginate from brown algae; agar and carageenan from red algae (McLachlan, 1985). These products are necessary auxiliaries in numerous industries as emulsifying agents, stabilisers, suspension-agents and thickeners (Heinz *et al.*, 1969; McLachlan, 1985).

The use of phycocolloids is wide spread and during the past three or four decades the industry has grown spectacularly comparable to the fast-food industry. The industry for phycocolloids such as carageenan and especially the low-sulfate type, furcellaran, was started as a result of World War II when traditional sources of agar was unavailable to the Allies (McLachlan, 1985).

Table 1. Present market value of seaweeds worldwide.

Product/Species	Market Value (US$/year)
Alginate *Macrocystis* sp., *Laminaria* sp., *Ascophyllum nodosum*, *Durvillaea* sp., *Lessonia* sp.	230 million
Agar *Gelidium* sp., *Gracilaria* sp., *Gelidiella* sp., *Pterocladia* sp.	160 million
Carageenan *Eucheuma* sp., *Chondrus crispus*, *Gigartina* sp., *Furcellarea lumbricalis*, *Hypnea* sp.	100 million
Seaweed meal *Ascophyllum nodosum*, *Fucus* sp.	5 million
Manure	10 million
Liquid fertilisers	5 million
Nori *Porphyra* sp.	1.8 billion
Wakame *Undaria* sp.	600 million
Kombu	600 million

(Jensen, 1993)

1.2 Economic Importance of Agar and *Gracilaria*

Phycocolloids derived from Rhodophyta include agar and carrageenan which form more or less firm jellies in aqueous solution. Agar, formerly called agar-agar, is a gel forming extract from certain Rhodophyta, the agarophytes that include *Gelidium Pterocladia* and *Gracilaria.* (Heinz *et al*, 1969; Doty and Santos, 1983). Agar is the oldest and perhaps the best known of all algal products. Agar production for many centuries was a world monopoly of the Japanese, and until 1939 Japan was the main producer. Agars are distinguished from carageenans by the β-1,4-galactose unit being in the L-form and in contrast to carageenan, most agars have low (<5%) sulphate content, and contain the agarose fraction (McLachlan, 1985).

Natural agars are consumed mainly in Asia in traditional cooking and come in conventional strips and square forms obtained from *Gelidium,* while in Japan, agar in tablets form are derived mainly from *Gracilaria.* Industrial agar are marketed as flakes, but mainly in powder form (Armisen, 1995).

Gracilaria consists of more than half of the world agarophyte tonnage and comes mainly from Chile. *Gracilaria* is cultivated on a very large scale in Chile, China, Taiwan for agar production while pilot scale cultivation is being carried out in medium-sized farms in Namibia, Venezuela and Mexico (Armisen, 1995). The important agar manufacturers are found in the USA, Japan, Denmark, Chile, Spain and France. Other than for microbiological purposes, *Gracilaria* is now considered to be the major raw material for the production of food and industrial agar (Jensen, 1993). The recent advancement in molecular biology contributed even more to the demand for high quality agar and agarose. Other industries in which agar from *Gracilaria* has been utilised include cosmetics, medical (as laxative), paper and fabric (Kim, 1970).

The supply of agar from *Gelidium* has not been able to meet the recent market demand and thus the importance of *Gracilaria* has emerged. In Taiwan and Japan, while *Gelidium* was the principal source of agar before 1960, *Gracilaria* has gradually replaced it. Among the important agar-producing *Gracilaria* species are *Gracilaria verrucosa* (Hudson) Papenfuss and *Gracilaria lemanaeformis*. The former species has become one of the most important raw materials of the Japanese agar industry (Doty and Santos, 1983). The production of *Gracilaria* agar in Chile has increased from 820 t to a current annual total 1320 t. In Indonesia, agar production from *Gracilaria* has increased from 150 t in 1984 to 450 t in 1993. Some factories in South Korea are now using *Gracilaria* as substitute for *Gelidium* (Armisen, 1995).

1.3 Tissue and Protoplast Cultures

Low natural populations of *Gracilaria changii* have prompted the use of micropropagation techniques such as tissue and protoplast cultures to regenerate new plants for mariculture. A variety of seaweeds have been cultured from their tissues or protoplasts.

Tissue cultures of *Porphyra umbicalis*, *Grateloupia dichotoma* and *Laminaria digitata* were shown to be successful in callus induction (Liu *et al.*, 1990; Yokoya *et al.*, 1996; Folefack and Cosson, 1995). Calluses induced from spores and vegetative fragments of *Gracilaria* spp. were successfully regenerated into new plants (Glenn *et al.*, 1996; Santelices and Verela, 1995). In general, protoplasts from a variety of seaweeds have shown to be totipotent in their ability to regenerate into multicellular microcolonies. Isolated protoplasts will generate cell wall in suitable culture medium, germinate and divide into polar multicellular embryos or clumps of microcolonies and ultimately germinate into a whole plant (Butler *et al.*, 1990). There have been few reports of successful regeneration of whole plants from protoplasts. Isolated protoplasts of seaweeds like *Bangia atropurpurea*, *Sphacelaria*, *Undaria pinnatifida*, *Enteromorpha intestinalis*, *Porphyra suborbiculata* and *Laminaria japonica* were shown to be able to regenerate only into the multicellular embryonic stage (Butler *et al.*, 1990; Butler and Evans, 1990; Toshiyaki *et al.*, 1994; Sawabe and Ezura, 1996).

Among the species of *Gracilaria* from which protoplasts were successfully isolated and divided are *G. tikvahiae*, *G. lemanaeformis*, *G. verrucosa and G.*

tenuistipitata (Cheney *et al.*, 1986; Mollet *et al.*, 1995; Corzo *et al.*, 1995). This is a research project on the tissue and protoplast culture of a local species of red seaweed, *Gracilaria changii*. *G. changii*, an agarophytic seaweed, is found abundantly in mangroves (Phang *et al.*, 1996).

1.4 Significance of Research

Due to low natural populations and seasonality of reproductive states of the local species of agarophyte, *Gracilaria changii* Abbot, Zhang and Xia, there is a need to regenerate them *in vitro* using tissue and cell culture techniques. Tissue culture allows regeneration of clones which are scarce in the natural habitat and at the same time induce somaclonal variations of the seaweed with different physical and biochemical characteristics. Cell or protoplast culture offers an even wider scope of advantages. Isolated protoplasts can be used as a model for fundamental biological studies and protoplast fusion (Burbidge *et al.*, 1994). Protoplasts are also ideal recipients of foreign DNA and larger particles such as organelles which facilitate the exchange of both intra-cellular and extra-cellular genetic elements as a basis for genetic manipulation, (Power and Chapman, 1987). This will lead to invention of novel plants. Production of high valued biochemical or bioactive compounds such as oxylipins by growing seaweed cell culture in bioreactor has been proven successful recently (Rorrer *et al.*, 1995).

1.5 Aims and Objectives of Research

This study is a preliminary investigation on the micropropagation of a local species of agarophyte, *Gracilaria changii* Abbott, Zhang and Xia (Rhodophyta) and has the following objectives:-

1. To develop tissue culture techniques for callus development and regeneration of *G. changii*.
2. To develop a simple and cost-effective protocol for isolating protoplasts from *G. changii*.