CHAPTER 1

INTRODUCTION

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1.1 Importance of Seaweeds

Seaweeds are used extensively as food and in industries. Two million tonnes of fresh weeds are processed annually to nori (*Porphyra* spp), wakame (*Undaria pinnatifida*) and kombu (*Laminaria* spp) and eaten, mainly in the Far East. About 1.5 million tonnes go to industrial production, mainly for the phycocolloids, alginate, agar and carrageenan (Jensen, 1993). When eaten as food, seaweeds supply the body with needed vitamins, essential minerals, important trace elements and iodine (Mshigeni, 1987). The sales of *Porphyra*, which is largely produced through cultivation in Japan, brings to the Japanese farmers an income of over US$ 2 billion annually (Mshigeni, 1987) with annual yields of nori sheets reaching 10 billion sheets in 1988. Meanwhile, the export trade of nori was valued at US$ 60 million in 1993 (Critchley, 1993a). The mariculture of *Porphyra* constitutes the most successfull venture of any cultivated marine product.

Seaweeds are also used extensively as agricultural fertiliser especially in Western Europe and North America. Two of the most widely used seaweed liquid fertilisers are Maxicrop and Alginure (Chapman, 1970). Some species of marine algae are also used as sources of medicinal products. *Digenia simplex* and *Chondria armata* are commercially used for the production of medicinal drugs (e.g. digesan) which are effective against the common intestinal worm *Ascaris lumbricoides* (Mshigeni, 1987).

One quarter of the global seaweed harvest is used for the extraction of phycocolloids (Luning, 1990). Alginates derived from large brown seaweeds are used predominantly in the textile (50%) and food (30%) industry. It can be sold for up to US$ 13 - 15.5 per kg (Critchley, 1993a). The main commercial sources of phaeophyte biomass are *Laminaria, Ascophyllum, Macrocystis* and *Sargassum.*

Carrageenan derived from the red algae include gel forming members as well as those which will provide viscosity and body to aqueous solutions and emulsions. Thus, they are used mainly in the food industry especially in dairy products and in bakery and confectionery commodities. The commercial sources of carrageenan are *Gigartina, Eucheuma* and *Chondrus crispus.*

Agar has been used as food in the Far East for several centuries. Agar is also extensively used in microbiology as a solidifying bacterial media. The demand for microbiological agars have increased due to new horticultural, entomological and genetic applications (Santelices & Doty, 1989). It is expected that the demands for agarose fraction of agar would remain high due to biotechnological and biochemical needs (Renn, 1984). The current use in tissue culture and as an electrophoresis medium is expanding (Critchley, 1993a). Because of its important uses, as well as the limited supply of the raw material, agar is currently the highest priced of all phycocolloids (Lavilla-Pitogo, 1992). In 1993, the world market value of agar was assessed at US$ 200 million (Critchley, 1993a). *Gelidium* species are the superior raw material in agar production but increasing quantities are coming from a number of *Gracilaria* species. The world's commercial agar comes mostly from gracilaroids (Hurtado-Ponce & Umezaki, 1988). The red seaweed *Gracilaria* is now the most important raw material and yields 60-70% of the world's agar (Guiry & Blunden, 1991).

Algae finds applications in many branches of modern industry (Chapman, 1970):

i) Food processing industries, e.g., bakery products, production of puddings, creams, jellies, cheese, salad dressing, fish, meat and fruit canning (as a protective coat against shaking during transport); clarification of wines, beers, liquors, etc.

ii) Cosmetic industries, e.g. as a stabiliser for emulsions in cosmetic creams, lotions, shampoos and ointments.

iii) Pharmaceutical industries, e.g., for coating pills and capsules.

Table 1: Main Commercial Seaweeds Worldwide and Their Uses

Seaweed	Extract	Main uses
BROWN SEAWEEDS		
Ascophylum *Durvillaea* *Laminaria* *Ecklonia* *Fucus* *Macrocystis* *Nereocystis* *Sargassum*	Alginate	Gelling, emulsifying and stabilising agents: food, paper, textiles, welding rods, pharmaceuticals, etc.
Same 8 species above		Fertiliser and animal feeds
Hizikia *Laminaria* *Undaria*		Direct human consumption
RED SEAWEEDS		
Gracilaria *Gelidium* *Acanthopeltis* *Pterocladia* *Gelidiella*	Agar	Gelling agent: foods (jellies, sweets, etc.). Bacteriological culture base
Chondrus *Eucheuma* *Gigartina* *Hypnea* *Iridaea* *Phyllophora*	Carrageenan	Stabiliser and gelling agent: confectionery, ice cream, meats, sauces, etc. Cosmetics and silk industry
Furcellaria	Furcellaran	As for carrageenan
Porphyra		Direct human consumption
GREEN SEAWEEDS		
Caulerpa *Enteromorpha* *Ulva*		Direct human consumption

(McHugh & Lanier, 1983)

1.2 Importance of *Gracilaria* Farming

The great bulk of agar is extracted from wild plants, and while the demand continues to increase, wild stocks of the algal sources are decreasing. *Gelidium* has an inherent slow growth (Wheeler *et al.*, 1981), therefore the need for cultivable alternative source of bacteriological grade agar is in order. Initial steps in *Gracilaria* farming has been undertaken by Doty (1978), and countries already practising *Gracilaria* farming include Chile, Japan, China and Philippines.

In addition, *Gracilaria* as food has become popular in the Caribbean and Hawaii with retail prices as high as US$ 6.00 to 6.50 per kg of dry *Gracilaria* in St. Lucia Island (Smith *et al.*, 1984) and up to US$ 3.50 to 5.00 per kg of wet weed in Hawaii. It is also used as a source of food for invertebrates in mariculture (Chiang, 1981).

Market demands for gracilaroids have increased markedly in recent years. This was evident with the eleven fold increase in *Gracilaria* exports from Chile during the period 1976 - 1983 (Santelices & Ugarte, 1987). This was due to a significant increase of seven and half times the FOB price in 1986 compared to the 1972 value of US$ 1500 per dry tonne (Santelices & Doty, 1989).

Market demands and lack of crop management have led to over-harvesting of the natural stands of *Gracilaria* (Santelices & Lopehandia, 1981; Smith *et al.*, 1984; Santelices & Ugarte, 1987) in several geographic areas. In 1989, the natural *G. gracilis* population at Saldanha Bay, Africa collapsed and no commercial collection were obtained for the following three years. The collapse of the Saldanha Bay population may have been the result of disease, since plants examined during subsequent partial die-offs of the Saldanha Bay beds exhibited symptoms such as lesions and thallus bleaching.

In addition, the pilot plant scale of *G. conferta* (Shousbe) J. et. Feldmann for the production of agar in IOLR (Israel Oceonographic and Limnological Research) has been facing the problem of disintegration disease (Friedlander & Gunkel, 1992). The major factors in thallus disintegration and bacterial infection of *G. conferta* by the 'white tip disease', were high temperatures, high plant density and lack of aeration. It is therefore no longer viable to depend only on the natural resources for economically important seaweeds. The proper know-how of seaweed farming and pest control is also necessary before *Gracilaria* farming is well underway. *Gracilaria* farming would then meet the demands for a product with reliable quantity and quality.

1.3 Principal Farming Areas

The principal farming areas are in the islands of Taiwan, Hainan in China and Chile. *Gracilaria chilensis* is cultivated in Chile. Exports for 1991, 1992 and 1993 were 1000, 1500 and 2000 dry tonnes respectively (Norambuena, 1996). *Gracilaria verrucosa* is farmed in Taiwan. Shang (1976) reported that an income of US$ 3.25 million annually was obtained through *G. verrucosa* farming in Taiwan. Advanced pilot farming has been completed in the U.S.A, St. Lucia Island in the Caribbean and Brazil. Farming is being developed in Thailand, Malaysia, Indonesia and Philippines (Santelices & Doty, 1989).

1.4 *Gracilaria* Farming Prospects In Malaysia

Natural populations of *Gracilaria* occur in inadequate quantities for commercial exploitation in Malaysia. *Gracilaria changii* are found in mangroves and areas free from active fishing practices. The first pilot culture programme of *Gracilaria* in Malaysia was carried out in 1983, in Penang under the Bay of Bengal Programme (Faazaz, 1986; Doty & Fisher, 1987). The species was *Gracilaria changii. Gracilaria changii* is known to produce good quality agar (Phang & Maheswary, 1989). An agar gel strength of 626.62 $g.cm^{-2}$ (16.84%) was obtained when the samples were treated with a combination of acid and alkali (Shaharuddin *et al.*, 1994).

Food grade agar which is consumed in Malaysia is imported mainly from Korea, Japan, Hongkong and China. In 1988, nearly 172 tonnes of agar worth about RM6.55 million were imported (Jahara & Phang, 1989). The demand for bacteriological agar is expected to increase in the coming years. In 1989, the bacteriological agar market in Malaysia was worth about RM2 million a year. There is no agar producing farm/factory in Malaysia.

The intensive mass-culture in farming that is required, as the initial and critical step of seaweed production, should ideally be free of diseases. Therefore, a checklist of such disease causing organisms forms an important basis for formulating disease control and proper management of seaweed mass culture systems. Currently, there is no information on the microflora of seaweeds in Malaysia.

1.5 Significance of Research

This study is intended to provide the necessary data for the formulation of disease control and management in seaweed farms. With the proper farming skills and disease control management, the seaweed *Gracilaria* can be cultivated on a commercial basis in Malaysia. Armisen (1995), made a study on *Gracilaria* imports into Japan from 1984 to 1993. In 1989, 20 tonnes were imported from Malaysia. There has not been any exports prior to that year and beyond it.

Gracilaria changii is already experimentally cultivated in Ban Merbok, Kedah under the Fisheries Department, Malaysia. Due to the abundance of this species in the mangroves and the fact that it is already farmed in Kedah, *Gracilaria changii* Abbott, Zhang & Xia was selected as the species to be screened for disease causing organisms.

1.6 Identification of Disease Causing Agents

In order to identify the causal agent of a disease, it is necessary to fulfill Koch's postulates (Black, 1996) which is :

1. The specific causative agent must be observed in every case of a disease.
2. The agent must be isolated from a diseased host and must be grown in pure culture.
3. When the agent from the pure culture is inoculated into healthy, but susceptible, experimental host, the agent must cause the same disease.
4. The agent must be re-isolated from the inoculated, diseased experimental host and identified as identical to the original specific causative agent.

Largo *et al.* (1995a) and Weinberger *et al.* (1994) demonstrated the pathogenecity of seaweed with a specific bacterial isolate by inoculating non-axenic algal culture. However, the above methodology was inadequate to identify bacterial pathogens of *G. gracilis* from Saldanha Bay, South Africa. The assay results were not reproducible, control plants developed disease symptoms and the bacterial strain inoculated into the algal culture could not always be re-isolated following the onset of disease. Lastly, the use of non-axenic *G. gracilis* resulted in bacterial contamination which prevented identification of a particular bacterial strain as the causative agent of the disease symptoms manifested by the inoculated algae (Jaffray & Coyne, 1996).

In this study, *G. changii* was used to screen for bacterial infection on axenic and non-axenic thalli. Axenic thalli were also used to screen fungal and viral isolates for pathogenicity according to Koch's postulates.

1.7 Objective

The project aims to establish information on disease causing organisms of *Gracilaria changii* Abbott, Zhang & Xia by:

1. Isolation and identification of surface and internal organisms, that is, bacteria, fungi, virus and algae, which are potentially disease causing in the seaweed *G. changii*.
2. Re-infection of healthy thalli of *G. changii* by bacterial, fungal and viral isolates to satisfy Koch's postulates.
3. Investigation of the effect of *G. changii* extract on bacterial and fungal growth.

4. Compilation of a checklist of organisms associated with disease symptoms in *G. changii.*