CHAPTER 2

LITERATURE REVIEW

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Seaweeds and other marine plants are important marine resources. They provide habitats for the spawning of marine organisms (eg. fish, molluscs, crustaceans, etc.), highly valued as food and are sources of gels and chemicals, used in everyday life. Natural resources of seaweed have been depleted due to over-harvesting and poor management of the habitats. Pollution has also led to deterioration of natural seaweed beds. This has made neccessary the farming of economically important seaweeds so that there is adequate supply for exploitation.

The main cultivated species of seaweeds are: *Laminaria japonica, Undaria pinnatifida, Eucheuma* spp., *Porphyra* spp. and *Monostroma rutidum. Enteromorpha* spp., *Caulerpa* spp. and *Cladosiphon okamuranus* are of recent and increasing economic value (Ohno & Critchley, 1993). *Eucheuma* is cultivated in the Philippines, Indonesia and Tanzania and *Gracilaria* in Chile.

In recent years, agar has become one of the many commercially important polysaccharides and the most widely known product extracted from red seaweeds such as *Gracilaria* spp. (53%), *Gelidium* spp. (44%), *Gelidiella*

spp. and *Pterocladia* spp. (remaining 3%) (McHugh, 1991). These species of seaweed belong to the Rhodophytes (red algae).

Wild stocks of *Gracilaria* spp., *Gelidium* and *Pterocladia* used to be extensively harvested from wild populations in countries such as Chile and South Africa for agar. However, due to the rise in demand and overharvesting of these seaweeds, seaweed farming was introduced to sustain a consistent supply to the world market.

Agar utilisation (consumption) was accidently discovered in the mid 17th. century and has been manufactured in Japan for more than 350 years (McHugh & Lanier, 1983). It was later developed in other Asian countries, South America and Africa. Agar has become a world commercial product. Agar consists of two major fractions, agaro-pectin and agarose where agarose has become a very desirable product of agar (Santelices & Doty, 1989). Agarose is used widely in biotechnology and biomedical science.

The most important characteristics of agar is its gel strength. Agar can be categorised into three groups based on this factor (Santelices & Doty, 1989).

1.   Sugar reactive agar. Agar gel strength is retained with the addition of sugar (at least 75 g per 100 ml in a 1% agar solution) and the gel becomes elastic. Sugar reactive agar is obtained largely from some

*Gracilaria s*pecies from the eastern Pacific: *Gracilaria lemaneiformis* (Santelices & Doty, 1989) and *Gracilaria chilensis* (Armisen, 1995).

2.   Standard agar which is normally used as a medium in microbiological purposes having a gel strength of above 600 $g.cm^{-2}$.

3.   Food grade agar with the lowest gel strength and specifications not meeting the above two.

In 1984, 7000 tonnes of agar were produced, of which approximately half was from *Gracilaria*, the remainder coming mainly from *Gelidium* (Coppen, 1989). The breakdown according to country was as follows (Table 2.1) :

Table 2.1 : AGAR: World Production, 1984 (tonnes)

Japan	2,440	Taiwan	275
Spain	890	Argentina	200
Chile	820	Indonesia	150
S. Korea	600	China	140
Morocco	550	Others	300
		TOTAL	6,685

Coppen (1989)

Within the countries of the Bay of Bengal Programme (BOBP), import figures given in Table 2.2 give some indication of the demand for agar within the region. The export figures, in Table 2.3, shows that very little is being exported.

Table 2.2 : AGAR IMPORT: Bay of Bengal Programme (BOBP)
Countries (tonnes)

Year	1980	1981	1982	1983	1984	1985	1986	1987
TOTAL	681	489	739	1,236	689	661	684	699
Of which by:								
Thailand	209	184	230	307	260	234	252	277
Malaysia	303	253	233	574	256	253	259	279
Indonesia	1159	43	262	350	163	170	165	140
India	6	6	4	3	6	3	5	NA
Sri Lanka	2	~(a)	7	~	NA	~	~	1
Bangladesh	2	3	3	2	4	1	3	2

(a) < 0.5; NA - Not available ; ~, negligible amounts
Coppen (1989)

Gelidium is the traditionally preferred source of the best quality agar and commands high prices. *Gracilaria* spp. tend to give good yields of agar but with poor gel strength. However, the discovery that alkali treatment improves the gel strength of agar process has increased demand for this genus.

Table 2.3 : AGAR EXPORTS: Bay of Bengal Programme(BOBP)
Countries (tonnes)

	1980	1981	1982	1983	1984	1985	1986	1987
TOTAL	2	15	6	15	33	40	24	19
Of which by:								
Thailand				~ (a)	1	12	12	11
Malaysia			2	12	10	9	12	7
Indonesia				2	1	1		
India	2	15	4	1	22	18	~	NA
Sri Lanka								
Bangladesh	~							

(a) < 0.5; NA - not available; ~, negligible amounts
Coppen (1989)

2.1 *Gracilaria* Farming

Farming of *Gracilaria* produces a crop that is more reliable in terms of the volume and quality of the product. A diversity of farming methods has been developed in *Gracilaria* farming. In open waters, basically three approaches are used for planting. They are bottom-stocking, nets or lines, and floating rafts. In bottom-stocking technique, vegetative thalli which are naturally attached to small stones and shells, are transferred to areas where an increased density is required. By 1985, sixty farms in Chile produced 410 tonnes of dry *Gracilaria* using this technique (Santelices & Ugarte, 1987). The problems encountered in

bottom-stocking include epiphytism particularly by *Ulva* and poor crop survival if the planting site is environmentally different from where the plants originated (Critchley, 1993b).

Vegetative thalli are tied to nets or lines which are then transferred to the mariculture site in another method. In the third technique, spores from reproductive thalli are allowed to settle onto ropes and when germlings are of sufficient size, the ropes are transplanted in the mariculture site. In Ceylon, by adopting rope farming techniques, 3.5 kg.m^{-1} could be obtained per crop. Raft farming in China yields about 2 tonnes.ha^{-1} dry material (Santelices & Doty, 1989). Raft farming of *Gracilaria gracilis* has been initiated at Saldanha Bay, South Africa (Jaffray & Coyne, 1996). "Problems encountered in line farming using vegetative thalli, include grazing by fish, sea urchins and molluscs, sediment accumulation and epiphytism. Some of these can be overcome through careful site collection, not allowing the crop to come into close contact with the bottom substrata or caging of the raft" (pg. 99 Critchley, 1993b).

Alternatively, *Gracilaria* can be farmed in ponds, raceways or tanks. *Gracilaria* is one of the largest seaweed to grow well in man-made ponds, which may have low salinity and water motion. *Gracilaria* has been cultivated in ponds, commercially and on a large scale in China and Taiwan (Critchley, 1993b). In China, ponds are reported to produce 2000 tonnes. y^{-1} dry *Gracilaria*

for agar production. Individual ponds of 0.7-1.0 ha in size, 60-70 cm deep have

temperature and salinity ranges of 15-30°C and 10-20 ppt respectively

(Santelices & Doty, 1989). Relatively high production figures of 40 tonnes. ha^{-1}

of dry material over a 150-day growing period were reported in China and

Taiwan (McLachlan & Bird, 1986). In 1985, a harvest of 1700 tonnes of dry

Gracilaria was obtained from Venice lagoon (Schramm, 1991). Several different

problems for example epiphytism, have been identified in the more advanced

farming operations in Taiwan. Taiwanese operators have developed a

polyculture system whereby several species of economically important marine

organisms are grown in the same pond at the same time. Epiphytism on

Gracilaria can be controlled through selective grazing by tilapia (*Oreochromis*

mossambicus) and prawns (Critchley, 1993b), as is done in Ban Merbok, Kedah

(Phang, 1998). The fish and prawns become a useful source of income to

farmers.

Tanks provide the greatest productivity per unit area and is the most

expensive form of cultivation compared to the other types of farming. The whole

process can be precisely controlled (e.g. water flow rate, nutrient and carbon

supply and aeration) and several steps may be mechanised, thereby reducing

labour input. Ryther *et al.* (1979) was able to produce *Gracilaria tikhvihiae* in

tanks with a mean productivity of 22 to 25 g dry weight m^{-2} day^{-1}. Tank

cultivation of *G. chilensis* (Ugarte & Santelices, 1992) and co-farming of

Eucheuma with *G. lichenoides* in the Philippines (Barraca, 1989) had also been successfully carried out. Higher costs entail due to this monitored system.

In Malaysia, *Gracilaria* has been successfully grown using bottom-stocking technique and line farming (Middle Bank, Penang). It is also grown in fishponds in Ban Merbok, Kedah (Fisheries Dept.).

2.2   Diseases of Seaweeds

Diseases and pests are major problems in the seaweed mariculture industry. The artificial nature of large monoculture cultivation systems provide ideal conditions for the spread of diseases and contaminants. Under natural conditions, genetic diversity among individuals of a population prevents the rapid spread of any disease, in the sense that the fittest survives. Therefore, the population will not be wiped out by a single infection of a disease. Under the artificial culture system, where they are more genetically uniform due to preselection for certain traits, the population would then be more vulnerable to infection and environmental variability.

Large monocultures provide ideal conditions for the spread of diseases and contaminants, whilst the growth under unnatural or limiting conditions will generally render the host plant more susceptible to attack. The three main

problems are: competition for space in the habitat, usually by fouling organisms; attack by pathogens such as bacteria, fungus and viruses and growth of algal epiphytes and endophytes. Diseases of seaweeds can be categorised into two types: that which is due to the environmental condition or that which is caused by infection by other organisms, the pathogenic diseases.

The environmental condition would tend not to be the most ideal in a farmed system due to its high stock density. There would be more intense competition for light, nutrients or the like. In a tank or pond culture, a delicate balance of the environmental factors maintained to as close to optimal as possible, is difficult in an outdoor farm or mariculture set-up. With natural environmental fluctuations, conditions would be less than optimal for the cultured species. Under such conditions of "stress" the plants will show signs of being "sick". Some of these systems include bleaching, loss of thallus rigidity and decay. The unhealthy parts of the thallus would then be subject to secondary infection by bacteria, fungi, other algae or invertebrates, eventually leading to plant death (Put-Ang, 1995).

2.2.1   Diseases Caused by Environmental Stress

The 'ice-ice' disease in *Eucheuma* and *Gracilaria* farms have been described as a disease brought about by unfavourable ecological conditions that

lower the resistance of the algae (Uyenco *et al.*, 1981). It is then susceptible to secondary infections by pathogenic microorganisms. Bacteria from the genera *Pseudomonas, Flavobacterium, Vibrio, Xanthomonas* and *Achromobacter* have been isolated from the unhealthy *Eucheuma* thalli. Pedersen *et al.* (1995) have described the 'ice-ice' disease as caused by an interplay of various ecological factors with the physiological state of the algae. In their study, 'ice-ice' disease was induced in both *Eucheuma denticulatum* and *Gracilaria cornea*. The alga under oxidative stress, produced very reactive and toxic compounds probably as a part of the algal defense system which can then become toxic to the alga itself.

Species of *Kappaphycus* and *Eucheuma* are important red algae cultivated in South-east Asia, both for the extraction of the phycocolloid carrageenan and as food. Largo *et al.* (1995a) concluded that unfavourable environmental factors cause the 'ice-ice' disease in the farmed seaweeds, *Kappaphycus* and *Eucheuma*. Light intensity of less that 50 µmol photon m^{-2} s^{-1} and salinity of $20^o/_{oo}$ induced 'ice-ice' whitening characterised by short segments at midbranches which were similar to those observed in seaweed farms in the Philippines. Temperatures of up to 33-35°C resulted in wide scale whitening of thalli. These effects were preceded by slow growth rates from an optimum of 3.7% d^{-1}.

The green disease of *Porphyra* has been reported to manifest itself at the time of substantial decrease in the nitrogen content of seawater (Lin & Lin, 1984). When the nitrogen content once again exceeds 40 mg.m^{-3}, *Porphyra* growth improves. Strong irradiations led to a higher rate of 'green disease' incidence as well.

2.2.2   Diseases Caused by Bacteria

Very little is known concerning bacterial pathogens of algae (Andrews, 1976; Andrews & Goff, 1984; Correa & Craigie, 1991) and *Gracilaria* spp. in particular. Bacterial invasion as a primary and secondary event in *Gracilaria* and other seaweeds, though few, have been reported. In a study by Andrew (1976), bacteria had been associated with algal galls, blights and rots. Frequently, the evidence is equivocal; the organisms isolated are not adequately separated and characterised; causality (Koch's postulates) is not established; or experiments *in vitro* cannot be extrapolated to natural conditions. Several cases of bacteria have been implicated. 'Black rot' of kelps of the genera *Macrocystis*, *Pelagophycus* and *Egregia* have been implicated as caused by bacterial infection. However, the causal organism was neither described nor classified (Andrews, 1976).

Later, agar degrading bacteria were found to be responsible for a 'white tip' disease and 'brown points' disease of *Gracilaria conferta* growing in

seawater ponds (Friedlander & Gunkel, 1992; Weinberger *et al.*,1994; Friedlander & Levy, 1995). Lavilla-Pitogo (1992) reported that agar - digesting bacteria were associated with 'rotten-thallus syndrome' in another *Gracilaria* sp. The bacteria were classified as belonging to the genus *Vibrio*. Agar-degrading bacteria normally form depressions or liquefaction of agar on plates (Austin, 1988). The cell wall of *Gracilaria* is composed primarily of agar. Agarases secreted by epiphytic bacteria may be responsible for disease symptoms such as thallus bleaching and lesion formation in this algal species (Jaffray & Coyne, 1996).

Low levels of nutrients such as phosphates is known to cause a certain species of *Eucheuma* to be more susceptible to bacterial disease (Uyenco *et al.*, 1981) . Unfavourable environmental factors such as suboptimal level of light intensity, temperature and salinity during laboratory culture induced the onset of bacterial disease in *E. denticulatum* and *K. alvarezii* (Largo *et al.*, 1995b). 'Ice-ice' disease was artificially triggered in these algae. The number of bacteria on and in 'ice-iced' branches were 10-100 times greater than those from normal, healthy ones. There was also an increasing proportion of agar-lysing bacteria in branches suffering from the 'ice-ice' whitening of *K. alvarezii* especially when subjecting branches to environmental stress such as reduced salinity and light intensity, suggesting that these bactera were occasionally pathogenic.

Rod-shaped bacteria have also been associated with a disease of the *Concochelis* phase of *Porphyra*. Galls on species of *Chondrus*, *Cystoclonium* and *Fucus* have also been observed. The galls which are proliferations of the inner (medullary) tissue contained bacteria in the intercellular spaces. The bacterium was isolated and partially characterised but it was neither classified, nor was there any indication of the purity of the isolates (Andrews, 1976).

'Green spot rotting' of *Porphyra* is an endemic disease of Japanese nori farms and has been attributed to *Pseudomonas* and *Vibrio*. Bacteria were isolated from infected fronds and reinoculated *in vitro* onto *Porphyra* fronds. Typical symptoms developed although the plasmoptysis which occurs in nature, did not appear (Andrews, 1976). Tsukidate (1983) found the non-pigmented bacterium *Beneckia* sp (=*Vibrio*) to be the causative factor of the 'white-rot' disease of the Japanese nori (*Porphyra leucosticta*). Kusuda *et al.* (1992) described a yellow bacterium, *Flavobacterium* sp., to be the cause of 'suminori' disease of the same seaweed. In Funka Bay , Hokkaido, Japan, the bacterium *Alteromonas* sp., was suspected to be the causative agent of the red spots disease of the cultured brown seaweed species, *Laminaria japonica* (Yumoto *et al.* 1989a, 1989b). A marine gliding bacterium, *Cytophaga* sp. was found to kill marine phytoplankton (Imai *et al.*, 1993). These diseases among cultured species were found to be associated with abnormal culture conditions, such as high temperature, high light intensity and low salinity, preceding a few days before the occurrence of these diseases.

Episodes involving algae, bacterial and fungal pathogens have been observed with *Chondrus crispus* in tank cultures (Craigie, 1995). It was observed that the bacterial invasion was a secondary event following wounding of the tissue.

2.2.3   Diseases Caused by Fungus

Fungi considered as algal pathogens are predominantly members of the Phycomycetes, Ascomycetes or Fungi Imperfectii (Andrews, 1976). Most algicolous marine fungi are Ascomycetes. The algicolous fungi are symbionts, parasites or saprobes. There has been a preference for lignicolous fungi as research objects over algicolous species, as most wood inhabiting fungi are ubiquitous by nature and easily accessible (Kohlmeyer & Kohlmeyer, 1979). Algae inhabiting fungi are relatively rare and limited in their geographic distribution to the range of their hosts.

As pointed out by Andrews (1976), studies on algicolous fungi have been undertaken mostly from a taxonomic approach, whereas few data on the host-parasite interactions, pathogenicity, predisposition and epidemiology are available. Parasitic fungi are restricted to three classes of algae: Chlorophyta, Phaeophyta and Rhodophyta (Kohlmeyer & Kohlmeyer, 1979). Some fungi attack a variety of genera within one class, as do, for instance, the Ascomycete *Lulworthia kniepii* and the Deuteromycete *Sphaceloma cecidii*.

Diseases caused by the Phycomycetes (Chytridiomycetes, Hyphochytridiomycetes, Oomycetes) have been reported (Sparrow, 1960). Phycomycetes occurs as saprophytes or facultative parasites on dead insects, algae, twigs and dead leaves and fruits (Jones, 1971). The 'red-wasting disease (Akagusare)' of *Porphyra* is caused by *Phytium porphyrae* (Sasaki & Sakurai, 1972). This disease has been very severe, destroying virtually an entire crop in certain areas within 2-3 weeks. Kazama and Fuller's (1973) ultrastructural study of *Porphyra perforata* infected with *Pythium macinum* Sparrow suggests that penetration is accomplished generally without the formation of appressoria or penetration pegs (Andrews, 1976). Host cells appear to plasmolyse when contracted by the pathogen, which invades inter- and intracellularly.

Petersenia lobata (Petersen) Sparrow, attacks various host eg. *Ceramium* and *Polysiphonia* species (Andrews, 1976). The pathogen is localised in the host sporangia where it kills developing mother cells. *Petersenia pollagaster* (Petersen) Sparrow was known to be a fungal parasite on the seaweed *Chondrus crispus* Stackhouse (Craigie & Correa, 1996). It causes the 'white rot disease' in tips in fronds of *C.crispus*. By attacking the meristematic regions of the fronds, this fungus stops growth and decreases the harvestable biomass. Scanning electron microscope observations have revealed that fungal zoospores settle on the host surface and penetrate the outer cell wall immediately, without an epiphytic stage. Subsequently, the fungal cell expands, becomes surrounded by a

cell wall, and undergoes multiple divisions to produce a mature sporangia consisting of depigmented, collapsed host cells surrounding each emptied sporangium. Yellowish-white lesions develop shortly after spores are released from the sporangium. It is not clear whether the final decay of the apices is due only to the fungal release of cytotoxins, enzymes, or both, or if it results from secondary bacterial invasion.

Kohlmeyer and Kohlmeyer (1979) have classified disease induced by Ascomycetes and Deuteromycetes into three groups based on symptoms in which: 1) the outer appearance of the host is unaltered; 2) discolouration appear; or 3) malformations are induced. 'Raisin disease' of *Sargassum* is caused by *Lindra thallassiae*. Infection is localised in the air bladders. No mention is made of the seriousness (Andrews, 1976). 'Black-dots disease' of the red alga *Gliopeltis* is caused by the perithecia of *Guignardia gliopeltidis*. The fungus does not induce any malformations in the host although fronds may be discoloured. *Alaria* and *Laminaria* have been known to be infected by the fungus *Phycomelaina laminariae*. The symptoms of this disease are small, localised, brown spots which rapidly expand and coalesce to form large necrotic or 'tar-like' areas. The ascomycete ramifies intercellularly throughout the peripheral cortex resulting ultimately in the disorganisation and death of large areas of the host and entry of secondary invaders.

Infection of calcareous red algae by *Lulworthia kniepii* (Ade et Bauch) Kohlmeyer are indicated by white spots with darkened centres due to the protruding necks of embedded ascocarps. Symptoms reminiscent of 'witches broom' of terrestrial plants have been observed in infections of red alga *Ballia callitricha* by *Spathulospora calva* (Kohlmeyer & Kohlmeyer, 1979) wherein hairs of the host proliferate and enclose the ascocarp of the parasite.

Shaumann and Weide (1990) identified three different species of fungi capable of decomposing alginic acid (a major cell wall polysaccharide) of the brown algae (Phaeophyta). They are the *Asteromyces cruciatus*, *Corollospora intermedia* and *Dendryphiella salina* and the enzyme responsible is alginase. These fungi thus are capable of decomposing algal material. Raghukumar *et al.* (1992), have isolated thraustochytrids and fungi have been isolated from the surface of six marine algae. Thrausthochytrids were isolated from *Centroceras clavulatum*, *Sargassum cinereum* and *Padina tetrastomatica* whilst mycelial fungi were isolated from all the above and *Ulva fasciata*, *Valoniopsis pachynema* and *Gelidium pusillum*. The effect on the growth of the marine algae was not studied. The pathogenecity therefore is not known. The extract of the seaweed *Sargassum cinereum* however, was able to promote the growth of the mycelial fungi (*Lindra thallasiae*).

Miller and Whitney (1981) found the fungus *Didymella fucicola* on the algae *Fucus vesiculosus* and *Mycosphaerella ascophyli* on the alga *Ascophyllum nodosum*. *Mycosphaerella ascophylli* is known to be an obligate endophyte of *A. nodosum* (Kohlmeyer & Kohlmeyer, 1979). In their study, the majority of the species isolated were terrestrial Fungi Imperfectii. Only *Cladosporium algarum*, *Dendryphiella arenaria*, *D. salina*, *Papulaspora halima* and possibly *Gliocladium roseum* (Pugh, 1974) could be described as marine. The species *Cladosporium algarum*, *C. cladosporiodes*, *C. herbarum*, *Penicillium ochrochloron* and *P. simplicissimum* were the most frequently isolated from living and cast seaweeds. Incidences of fungus as a pathogen in *Gracilaria*, however have not been cited.

2.2.4   Diseases Caused by Virus

In contrast to the rich knowledge of viruses on terrestrial plants, virus parasitising algae, although known to exist since the early 1970s, have been poorly studied. Since 1972, there has been a series of reports concerning viral-like 'infections' of marine algae (Andrews, 1976). Although the anomalous particles have been called viruses by some authors, there is no solid evidence yet. Several freshwater algal viruses have already been characterised (Gibbs *et al.*, 1975).

The virus-like particles may vary in size, but have certain features in common including shape, location in or near the nucleus, and in the case of benthic algae, occurrence in sporangia or zoospores (Andrews, 1976). Characteristically, they appear to consist of an outer dense envelope that encloses an electron dense envelope within a lighter matrix. Their physical appearance and cellular location are similiar to those of herpes viruses of animals (Cook & Stevens, 1970). These putative virions are generally associated with varying degrees of cellular derangement such as disruption of the nucleus and dictyosomes (Markey, 1974) or degeneration of mitochondria and chloroplast (Toth & Wilce, 1972). There is no evidence of how dissemination of these particles in the marine environment occurs but it is presumably through wounds or possibly pinocytotic uptake by naked algal zoospores.

The ESV-1 virus is known to infect zoospores of *Ectocarpus siliculosus, Feldmannia simplex* and *Kuckuckia kylinii*, which is a closely related genus (Muller, 1996). The resulting *Feldmannia* plants were sterile and had on their assimilatory filaments inflated lateral appendages that appeared highly vacuolated. Electron microscope examinations showed disorders in the fine structure of these cells, but no presence of virus particles.

Virus was found to infect three marine brown algae: *Feldmannia irregularis, F. simplex* and *Ectocarpus siliculosum* (Muller & Frenzer, 1993).

Virus particles were produced in deformed reproductive organs (sporangia or gametangia) of the hosts and are released into the surrounding seawater. New infections occur when gametes or spores get in contact with virus particles. The virus genome enters all cells of the developing new plant via mitosis. Virus expression is variable and in many cases the viability of the host is not impaired. Infected host plants may be partly fertile and pass the infection to their daughter plants. Meiosis of the host can eliminate the virus genome and generate healthy progeny. The genome of the *Ectocarpus* virus consists of dsDNA. Meiotic segregation patterns suggest an intimate association between virus genome and host chromosomes. An extra-generic host range has been demostrated for the *Ectocarpus* virus. Field observations suggest that virus infections in ectocarpalean algae occur on all coasts of the world, and many or all *Ectocarpus* and *Feldmannia* populations are subject to contact with virus genomes. The filamentous marine brown alga *E. siliculosum* and *F. simplex* is infected by host specific virus which are only expressed in the reproductive cells but their vegetative growth is rather normal (Friess-Klebl, 1995).

Virus was also found to infect *Chlorella* and *Chlorella*-like algae (Van Etten *et al*., 1991). The *Chlorella*-virus (PBCV-1) enters its host by lysis of the cell wall. In contrast, the viruses of the filamentous brown algae *Ectocarpus* and *Feldmannia* infect their hosts only by entering their flagellated zoospores or gametes. The infected plants grow up to somatically normal plants. Symptoms

become visible and virus particles are formed only in the prospective reproductive organs of the plants.

The haptophyte *Phaeocystis pouchetii* (Hariot) Lagerheim isolated from Norwegian coastal waters in May 1995 was found to be infected by a host-specific virus (Jacobson, 1996). Cell lysis was the major loss factor for *Phaeocystis* during the decline of a bloom in the North Sea, accounting for 75% of the decrease in cell abundance. This indicates that lytic viruses may play a key role in termination of algal blooms. Infection by viruses or virus-like particles have never been described for any marine alga of Rhodophyta genus.

2.2.5   Disease Caused by Epiphytic Algae

A major problem in the outdoor cultivation of *Gracilaria* sp. is the development of competing epiphytes. Epiphytes can reduce the amount of light reaching the host algae, compete for nutrients and gases dissolved in the seawater, add weight to the host algae promoting the detachment of the entire, or part of the host algae (Enright, 1979; Sand-Jenssen *et al.*, 1985; Kuschel & Buschmann, 1991) and/or by releasing exudates that can promote, or be detrimental to the host algae (Harlin, 1987; Pedersen *et al.*, 1995). Tissue damage to the host resulting from the penetrating activity of the attaching rhizoids of the epiphytes, can also be a problem; this becomes further

exacerbated if attempts at hand removal of epiphytes are practised. Figure 1

shows an example of an epiphyte attached to the thalli of *Gracilaria changii*

isolated from Morib.

Fig. 1. An epiphyte attached to the thalli of *G. changii*.

Santelices and Ugarte (1990) have noted significant inter-strain

differences in susceptibility to epiphytes (*Ulva, Enteromorpha* and *Ectocarpus*).

This was interpreted to represent stimulation of epiphytic attachment by the

thalli of *G. chilensis*. Additions of various concentrations of soluble fraction of

agar extracted from dry thalli of *Gracilaria chilensis* to the culture medium

resulted in statistically significant increases in the density of settled spores of *E.*

compressa and *U. rigida*. *Ulva* can significantly depress *Gracilaria* biomass production adding weight to the host algae consequently leading to the dislodgement. These appear to be the main mechanism involved in the *Ulva-Gracilaria* interactions (Buschmann & Gomez, 1993). *Ulva* is one of the commonest algal species identified as epiphytes reaching loads of 60% g of epiphyte per g of *Gracilaria* in the intertidal cultures of Southern Chile. The main epiphytes identified in the *Gracilaria conferta* (Schousbe) J. et. Feldmann outdoor cultivation tanks were *Enteromorpha compressa* Greville, *Cladophora pellucida* Kuetzing, *Ulva lactuca* Le Jolis and *Ectocarpus confervoides* Le Jolis (Friedlander, 1992). Under natural conditions (40 ppt, 25°C), the major potential competitor for *Gracilaria conferta* was *Ulva lactuca* while both *Enteromorpha intestinalis* and *Ectocarpus confervoides* shared competition under suboptimal conditions (Friedlander, 1992).

In spring 1993, a bloom of *Ulva lactuca* appeared for the first time in Saldanha Bay, South Africa and persisted through summer. Ammonium-rich fish-factory discharge into a sector of the bay provided localised conditions for *Ulva* to out-compete *Gracilaria* at depths of 2-5 m (Anderson *et al.*, 1996). With N-enrichment and sufficient light, *Ulva* has been shown to outgrow *Gracilaria* species (Fujita, 1985).

Phytoplankton, brown algae and red algae of which there are at least 40 genera are known to parasitise other algae (Andrews, 1976). Symptoms of red algal parasites are generally restricted to a localized swelling or the presence of the parasite itself. Red algal parasites that are closely related to their host are called adelphoparasites; those showing no close relationship, alloparasites. A striking fact is that about 90% of the former assemblage belong to the same family or order as their host (Andrews, 1976).

In Japan, Yamamoto (1986) described an adelphoparasite red alga, *Congracilaria babae* which grows on *Gracilaria salicornia* (C. Agardh) Dawson. At the beginning of growth, the parasite can be recognised as a mere swelling on various places on the host plant. Upon maturation, it becomes spherical and then develops into a mushroom-shaped structure with a short stipe. Rhizoids penetrating the host were not recognised though tissue between parasite and host appear continous via pit connections (Yamamoto, 1991). Yamamoto and Phang (1995), discovered an adelphoparasite having physiological similarities to the *Congracilaria,* which induces similar symptoms in the host *G. salicornia*. The parasite was observed in specimens obtained from Morib (Selangor), Malaysia.

Sturch (1926) proposed that certain red algal parasites now known as alloparasites originated from epiphytes (i.e. plants using other plants only for

mechanical support) by gradually penetrating the host tissues and establishing a metabolic dependence. The initial events in pathogenesis that has been documented is the attachment of spores to the host surface by a mucilaginous disc and germination to produce a short filament which characteristically has a swollen tip and may also adhere by mucilage (Rawlence & Taylor, 1972). Penetration is intercellular or intracellular. Rawlence (1972) has presented cytological evidence, such as a change in the host wall structure and degeneration of its cytoplasm, that it may be mediated enzymically. In the filament, there is a large increase in dictyosome vesicles which discharge at this time, concomitantly with an increase in wall thickness. These events are reminiscent of the stages in penetration of terrestrial plants by higher fungi (Andrews, 1976).

Goff (1975) showed that ^{14}C radioisotope was assimilated by the host plant and transferred primarily from medullary cells to the adjacent rhizoids of the alloparasite *Harveyella mirabilis,* demonstrating the detrimental effect of an algal parasite to the host.

2.2.6   Diseases Caused by Endophytic Algae

Kelps are harvested for the commercially important extractive, alginates. Among the brown algae, various galls, apparently induced by *Streblonema*

appear on the stipes and blades of kelps, as seen on *Nereocystis integrifolia* (Andrews, 1976). *Streblonema* is an endophytic brown algae (Yoshida & Akiyama, 1979) and is common in kelps. Apt (1988) provided for the first time experimental evidence that *Streblonema* sp. can cause hyperplasia in *Nereocystis*, *Macrocystis* and *Laminaria*. Epidemiological data of *Streblonema* infections were shown by Andrews (1977) whereby infected individuals of *Nereocystis* at San Juan Island (NE Pacific) increased from 20% in June to 90% in August. Meanwhile Yoshida and Akiyama (1979) reported wild Japanese *Undaria pinnatifida* were infected often by *Streblonema aecidioides*. The resulting texture changes decrease the commercial value of *Undaria*.

Peters and Schaffelke (1996) found *Streblonema* to infect the brown alga *Laminaria saccharina* in Kiel Bay, Western Baltic. Alterations of blade and stipe, range from dark spots to heavy deformations and completely crippled thalli. The red alga *Chondrus crispus* Stackh., Irish moss, occurs in Europe from Northern Norway south to North Africa. It is of commercial importance due to its extract, carrageenan. Nielsen and McLachlan (1986) reported the green filamentous species *Endophyton ramosum* Gardner as endophytic in fronds of *Chondrus crispus* from the western Atlantic.

Correa *et al.*, 1987 reported green and brown endophytes which occur together in fronds of *Chondrus crispus*. The infection was observed to spread

from one frond to another by spores discharged by the endophytes into the surrounding medium. The endophyte, once within the frond, has only limited capability of spreading by vegetative growth. Many of the heavily infected fronds are broken and this may indicate frond fracture following tissue weakening caused by endophytes. This view is supported by observations on laboratory-infected fronds of *Chondrus crispus*; these undergo significant cellular damage during infection by either *Acrochaete operculata* Correa et Nielsen or *A. heteroclada* Correa et Nielsen (Correa, 1990), the two most common green endophytes recorded for this host.

In a later study by Correa and McLachlan (1992), when the seaweed *Chondrus crispus* was infected under laboratory conditions by the green algae *Acrochaete operculata* and *Acrochaete heteroclada*, detrimental effects on the host performance including slower growths and lower carrageenan yields resulted. Infections also caused softening of host tissue. Infected *C. crispus* were preferred by crustacean grazers. These endophytes also acted as agents facilitating secondary infections by other pathogens including bacterial invasion. Craigie and Correa (1996) showed the interrelationship leading to the disease of cultivated *C. crispus*. Wounding was initiated by grazing and the germination of spores of fungal pathogens *Acrochaete* and *Petersenia* on the surface of *C. crispus*. Upon sporogenesis, both pathogens cause death of localised cortical cells   with   concomitant   invasion   of   the   bacteria   from   the

Cytophaga/Flavobacterium group found in the wounded tissue. The pathogenecity depends on the chemical constituents of the host.

Correa *et al.*, 1994 reported a 'green patch disease' in *Iridaea laminaroides* (Rhodophyta) caused by *Endophyton* sp. (Chlorophyta). The disease is widespread along the coast of central and Southern Chile. The disease is characterised by a green mottled colouration, softening of host tissue due to endophyte-mediated compaction of cells, followed by secondary bacterial infections.

2.2.7   Diseases Caused by Other Organisms

Whitening of *Gracilaria chilensis*, accompanied by tissue softening and thallus fragmentation was found to be associated with the presence of an endophytic amoeba (Correa & Flores, 1995). Although the symptoms developed originally in green mutant thalli, subsequent infections in the laboratory also affected normal wild-type *G. chilensis*. Ultrastructural evidence indicates that the amoeba perforate the host cell walls of both cortical and medullary cells and digest their protoplasm. Destruction of the host tissue resulted in large cavities first, followed by thallus fragmentation. The amoeba isolated was from the class Rhizopoda. Another amoeba isolated was from drift *Sargassum muticum* which was capable of fully degrading algal cell walls and cuticles (Polne-Fuller &

Gibor, 1987). Protozoas, nematodes and copepods have been reported to some algal pathologies characterised by malformations and decaying lesions, but no experimental evidence exists so far to support the notion that these organisms are primary pathogens (Andrews, 1976). Hanisak (1987) noted that fouling diatoms on the sides of culture ponds detached, entrapped and caused *Gracilaria* to float, making the plants vulnerable to the high surface summer temperature.