PERSPECTIVES IN MARICULTURE

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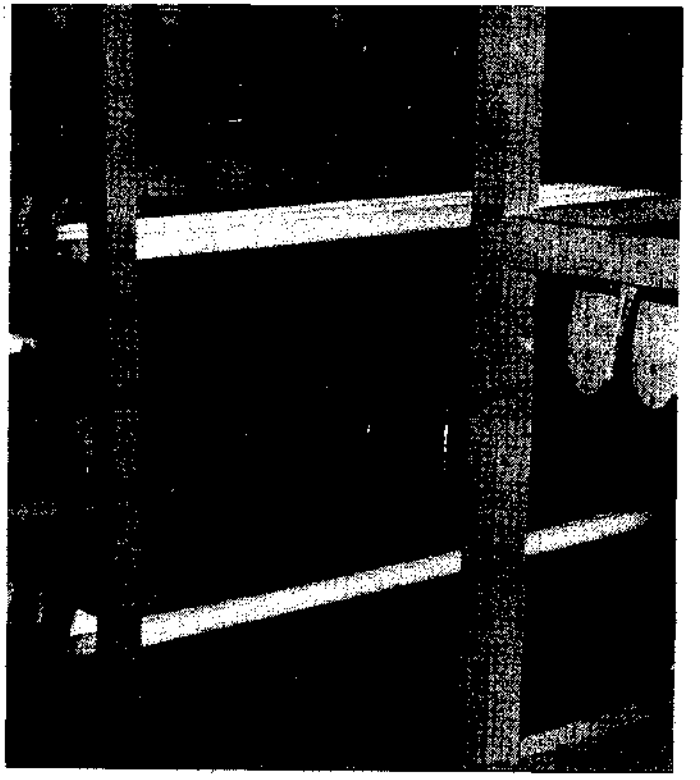

Prospects of biotechnology in seaweed mariculture in India

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ABSTRACT

Red seaweeds are the major source of economically important colloids, agar and carrageenan. Agar industry in India shall become commercially attractive only when the yield of agar from the raw material is enhanced. Inherently most Indian agarophytes contain 10-20% agar only. Though species of Gelidiella *and* Gelidium *contain 35-50% agar and the quality of agar in terms of gel strength is also superior, their biomass production is very less, occurrence is seasonal and their exploitation is difficult. Hence an attempt is underway to obtain a hybrid strain of red seaweed for large scale mariculture between slow growing but high agar containing seaweed and fast growing, poor agar yielders. This article enumerates the prospective areas of seaweed biotechnology and its immediate relevance to seaweed mariculture and the related industry in India.*

Introduction

Polysaccharides produced by seaweeds form the basis of an economically important and expanding industry such as agars, agaroses, algins and carrageenans, which are used as ingredients in food, pharmaceuticals and many other industrial and consumer products. Seaweed biotechnology has got to its credit the following prospective areas:

1. Production of genetically modified strains of commercial seaweeds.
2. Acclimatization, culture and propagation of exotic varieties of seaweeds under controlled conditions.
3. Repositories of seaweeds whose occurrence is seasonal or rare and are facing the threat of extinction due to indiscriminate exploitation.
4. Bioremediation of toxic pollutants in sea and land based mariculture systems and
5. Extraction, isolation and manipulation of bioactive peptides, polysaccharides, metabolites and drugs from marine micro and macro algae that are maintained in *vitro* conditions.

Of these aforelisted prospective areas, production of genetically modified seaweed strains has the direct and immediate relevance to the seaweed mariculture and their industrial utilization, as the Indian agar yielding seaweed species yield only around 10-20% agar and possess low gel strength (100-150g/cm^2; Anon, 1995). Hence, agar industries in India can't function economically viable. To make the industry commercially attractive, a raw material that can promise better yield and superior quality is the only prerequsite. Though species of *Gelidiella* and *Gelidium* contain 35-53% agar and the quality of agar in terms of gel strength is also superior (350-400 g/cm^2), their biomass production is very less (Wheeler *et al.*, 1981) as well as seasonal and as they are firmly attached to the rocky substratum, their exploitation is difficult. Hence an attempt has been made to obtain a hybrid strain of red sea weed for large scale mariculture, between crustose thalloid but high colloid containing

seaweed and fast growing foliose type poor-yielders through genetic modification of seed stock (Kaladharan, 1998).

Protoplasts as seed stock

For all current methods of macroalgae culture upto 25% of the cultivated material is required as the seed. This reduces productivity and makes the cultivation efforts labour intensive, where the seed stock is attached to the culture rafts by hand. Instead protoplats could be used as seed stock for seaweed mariculture (Renn, 1977). Significant progress in obtaining viable protoplasts has been reported for *Porphyra linearis* (Chen *et al.*, 1994), other species of *Porphyra* (Polne-Fuller and Gibor, 1986), *Gracilaria tikvahiae* (Cheney, 1984), *Gracilaria verrucosa* (Belleanger *et al.*, 1990), *Chondurus crispus* (Le gall *et al.*, 1990), *Kappaphycus alvarezi* (Zablackis *et al.*, 1993), *Gelidium sp* (Gusev *et al.*, 1987), *Pterocladia capillocea* (Liu *et al.*, 1990) besides many other species of green and brown seaweeds (Butler *et al.*, 1990). Techniques and conditions of protoplast isolation from seaweeds, their characteristics in *in vitro*, their culture and fusion were reported earlier (Kaladharan, 1999).

Genetic modification of seaweeds

Methods for the introduction of foreign genes into other organisms include protoplast fusion; introduction of the desired genes by appropriate vectors, including insertion of transformed plasmids; infection with transformed plasmids; infection with transformed viruses or other specific pathogens; and direct insertion of the gene by electroporation or ballistics. Protoplast fusion in seaweeds can best be achieved either by electrofusion through cell fusion system (Mizukami *et al.*, 1995) or chemically induced by Poly ethylene glycol (PEG). Somatic cells from the meristematic thallus of gametophytes (n) are isolated and viable protoplasts are isolated through enzyme treatment. These protoplasts are allowed to fuse each other and karyogamy is induced. From the diploid callus through morphogenic induction tetraporophyte (2n) can be developed (Fig. 1).

Development of interspecific hybrids of *Gracilaria*

a) *Gracilaria edulis vs G. verrucosa*

G. verrucosa grows in quiet warm shallow bays and in brackish waters throughout the year. Thus fusion of protoplasts from gametophytic thalli

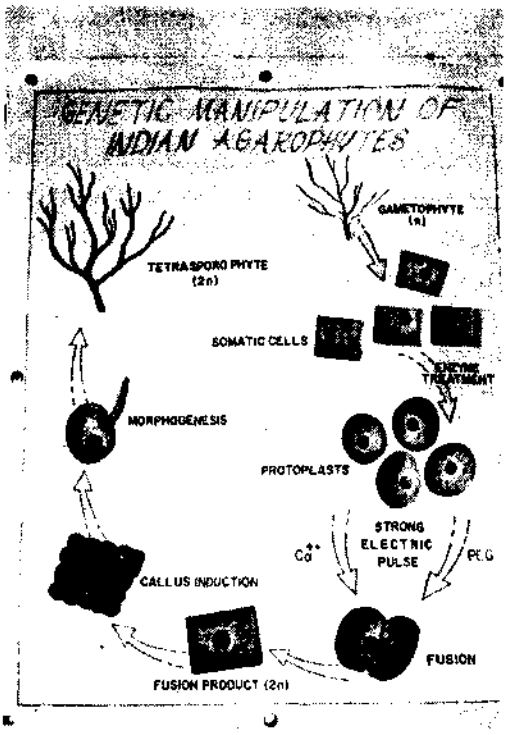

Fig. 1 : Schematic representation of hybrid strain of tetrasporophyte of *Gracilaria edulis* production through protoplast fusion

of *G. edulis* and *G. verrucosa* can result in a fusion product that can be regenerated to a new strain with improved agar and gel strength. This may be an ideal strain for brackish water habitats, as brackish water areas are the most suitable for large scale mariculture of seaweeds, integrated with bivalves and shrimps.

b) *Gracilaria corticata vs G. edulis*

Distribution of *G. edulis* is restricted to Tuticorin, Mandapam, Lakshadweep and Andaman-Nicobar Islands in India. Whereas, *G.corticata var. corticata* is widely distributed all along the Indian coasts especially the west coast (Anon. 1995). Hence the hybrid strain obtained through para sexual fusion would be a promising clone for large scale mariculture along the coasts of Kerala and Karnataka.

c) *G. edulis vs Gracilaria NBr 10*

Nova Biosciences of the Philippines has developed a fast growing subtropical strain termed *Gracilaria NBr-10* and successfully propagated in the laboratory and transferred for expansion of culture at Nova's Biological Research Station located at the Science and Technology Park of the University of Phillipines, Iloilo. Besides being a fast growing strain, *Gracilaria NBr-10* has a high agar content (18%) and a gel strength of 680-950 g/cm^2 (Anon, 1996). A somatic hybrid between *G. edulis* and NBr-10 would be suitable for tropical climate and can be cultivated in

ponds, raceways and other land based culture systems to get a highly priced product having higher gel strength.

Intergeneric hybrid between *Gelidiella acerosa* and *Gracilaria edulis*

Species of *Gelidiella* and *Gelidium* are known to contain high content of agar (45-53%) with higher gel strength (350-425 g/cm^2). An intergeneric hybrid produced either through fusion of protoplasts or through insertion of gene responsible for agar content into *G. edulis* either through vectors or mechanical means would make the seaweed mariculture and agar industry commercially attractive.

Gene mapping, sequencing and transformation

A prerequisite for genetic engineering is an adequate understanding of genome structure, sequence and gene expression. Although the homology is not complete, probes from land plants, unicellular algae and other micro organisms have been used with success to locate specific genes. Considerable work has been done using unicellular and green algae and Cyanobacteria, but reports of mapping and sequencing red marine microalgal genomes are scarce. The chloroplasts ribosomal-protein-encoding genes of the agarophyte, *Gracilaria tenuispitata* have been located, cloned and characterized (Kao *et al.*, 1990). A 1365 bp region around this gene was also sequenced; the gene order was found to be identical to that detected in the chloroplast DNA of liverwort, tobacco and maize. The plastid gene for the rp 122 protein in *Gracilaria tenuispitata* has also been isolated and sequenced (Kao and Wu, 1990).

The polymerase chain reaction (PCR) has been used to amplify *Gracilaria pacifica* nuclear and plastid ribosomal genes from algal herbarium specimens and spores (Goff and Moon, 1993). The glyceraldehyde 3 phosphate dehydrogenase gene system of the red alga *Chondrus crispus* has been investigated (Liaud *et al.*, 1993). Promoter structures, intron/exon organization, genomic complexity, differential expression of genes and transcript level of the genes in the gametophytes and the protoplasts were determined, paving the way for genetic transformation in *C. crispus*

Five of the twenty on red algal genera studied by Goff and Coleman, (1990) were found to contain circular double stranded DNA plasmids. Some

of these were isolated and one 3.0 kbp plasmid from *Gracilariopsis lemaneiformis* was sequenced to reveal two potential open reading frames. In this species, plasmids are present in a high copy number/cell and may provide useful vectors for algal transformations. The DNA sequence and structural organization of the GC2 plasmid from the agarophyte, *Gracilaria chilensis* has been determined (Vilemur, 1990). This 3827 bp circular plasmid has one major open reading frame that generates a transcript and could encode a 411 aminoacid polypeptide. Henry and Meints (1994) suggests that recombinant viruses, particularly the large ds DNA viruses that are known to infect eukaryotic algae can be used as transformation vectors for marine microalgae.

Conclusion

The polysaccharides from seaweeds are the basis of well established growing industries. Many consumer products rely on their unique properties and would not exist without their availability. With increased research emphasis and wide applications possibly even new polysaccharides from new seaweed strains might emerge. Genetic understanding and techniques for the introduction of foreign genes are evolving. On the whole the prospect of biotechnology are bright to seaweeds, their mariculture and polysaccharides they produce.

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