

Biotechnology and the red seaweed polysaccharide industry: status, needs and prospects

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Significant amounts of marine macroalgal (seaweed) polysaccharides are used in food, pharmaceuticals and other products for human consumption. Thus, the global seaweed polysaccharide industry operates in a highly regulated environment. Genetic manipulation of macroalgae to alter composition or growth characteristics may lead to products that do not fall within the current regulations: research that is readily translatable to industrial application is generally restricted to seaweed cultivation and processing and new applications of the approved polysaccharides. There is a great need, however, for research into the genome structure and metabolic pathways of commercially important marine macroalgae. This pre-competitive research may not be immediately applicable to the seaweed polysaccharide industry but is critical for sustaining future commercial growth.

Polysaccharides produced by marine macroalgae (seaweeds) form the basis of an economically important and expanding global industry^{1,2}. Key products are agars^{3,4}, agaroses^{3,5}, alginates^{6,7} and carrageenans^{8,9}. These are used as ingredients in food, pharmaceuticals and diverse consumer products and industrial processes (Box 1). Despite the potential for eventual commercial application of basic research into the biology of marine macroalgae, and subsequent alteration of biosynthetic pathways, the seaweed polysaccharide industries have been slow to accept changes and have, therefore, given minimal support for this research. Economic considerations are probably the main factor determining this attitude. The majority of commercial polysaccharide products have existed for many years with the same specifications. Companies are reluctant to alter those long-accepted extracts and blends and the raw materials from which they are recovered. The regulatory hurdles associated with the introduction of novel products or processes, coupled with an overriding need to minimize production costs in a competitive industry, mean that the majority of ongoing commercial research targets the development of alternative, less costly production methods and new applications for established products rather than more

fundamental studies. This article considers the current status of basic research, focusing on progress in the application of molecular biology techniques to macroalgae. Integration of a better understanding of this molecular biology into current R&D strategies could enable translation of basic research into new products, processes or sources of raw materials.

Research and development on the marine macroalgae and the polysaccharides they produce may be considered in two categories: (1) basic and applied work supporting the immediate and short-term needs of the industries; and (2) long-term R&D.

Short-term R&D

To reach the present sustainable, cost-effective status of seaweed cultivation, key areas of research have been: species and strain selection, vegetative reproduction, methods and locations for cost-effective cultivation, timing of and methods for harvesting, and optimizing drying and storage conditions. Processing the macroalgae to obtain the polysaccharides in appropriate forms for commercial utilization has evolved over many years with the origin of many key steps dating back over half a century. Research and development has been required to optimize the yields and assure product quality. In addition, process modifications have been, and continue to be, needed for matching product properties to new applications. This R&D has been reviewed previously^{3,4,8,9}.

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Box 1. Commercially important seaweed polysaccharides

Although some marine macroalgae, or seaweeds, are eaten as food, particularly in the Asian-pacific region, and other biochemicals derived from them have small niche markets, their major economic value is associated with the polysaccharides certain species contain. Algins, carrageenans and agars have all achieved commercial significance because of their food and industrial applications and are the basis of an estimated billion dollar global effort^{1,2}. This encompasses all segments of activity, from gathering wild harvests of the seaweeds, cultivating and collecting important varieties, processing, to preparing finished products containing the polysaccharides. Each of the major polysaccharides, as well as products derived from them (e.g. agaroses from agars), also has important life sciences applications^{46,47}. For example, a number of the significant advances in modern biotechnology are based on the availability of various types of agarose. Immobilized enzyme reactors frequently rely on microorganisms encapsulated in calcium alginate or potassium κ -carrageenane gel. Insulin-producing pancreatic cells encapsulated in calcium alginate and implanted in patients' abdomens are keeping previously incapacitated diabetics alive and active. Carrageenans are used in medical research to induce a particular response in model systems and are being used in screening assays for new drugs. In particular, the rat-paw edema assay has led to the development of many of the currently used anti-inflammatory drugs.

The four major classes of marine macroalgae are Rhodophyta (red algae), Phaeophyta (brown algae), Chlorophyta (green algae) and Cyanophyta (blue-green algae). Only the red and brown macroalgae are currently sources of polysaccharides of significant commercial value. The seaweeds from which these polysaccharides are extracted are harvested from their natural growth, gathered from beaches or collected from cultivated beds. Carrageenans^{8,9} and agars^{3,4}, from which agaroses^{3,5} are derived by purification, are obtained from different genera of red algae. Algins^{6,7} are obtained from a number of species of brown algae and are present in all. Tables 1 and 2 summarize the sources, compositions, properties and more important applications of these polysaccharides. Although a number of competitive gelling and viscosifying hydrocolloids exist, the unique properties of the seaweed polysaccharides are the sustaining force behind this industry.

Applications R&D is essential to develop new markets for seaweed polysaccharides. Changing consumer requirements and competitive markets drive R&D. One of the present driving forces is low-fat/low-calorie food products. Research directed towards

elucidating the chemical composition of the various marine macroalgal polysaccharides has been important in seaweed selection and processing and finished product applications. Feeding and toxicology studies are assuring the safety of the ingested polysaccharides.

Table 1. Seaweed polysaccharides: sources and compositions

| Polysaccharides | Important raw materials (genera) | Composition |
|--------------------|---|---|
| Agars and agaroses | <i>Gelidium</i> , <i>Gracilaria</i> , <i>Pterocladia</i> , <i>Gracilariopsis</i> , <i>Porphyra</i> | Alternating 1,4-linked α -D-galactose and 3,6-anhydro- α -L-galactose backbone (agarobiose) substituted with varying percentages of methoxyl, ester sulfate and ketal pyruvate groups |
| Algins/alginate | <i>Macrocystis</i> , <i>Laminaria</i> , (Microbial: <i>Azotobacter vinelandii</i> , <i>Pseudomonas</i> sp.) | 1,4-linked α -L-guluronic acid and β -D-mannuronic acid subunits in GG, MM and MG domains |
| Carrageenans | | <i>kappa</i> - and <i>iota</i> - Alternating 1,3-linked α -D-galactose and 1,4-linked 3,6-anhydro- β -D-galactose backbone (carrabiose) substituted with varying percentages of ester sulfate |
| <i>kappa</i> - | <i>Eucheuma (cottonii)</i> , <i>Kappaphycus (alvarezii)</i> , <i>Gigartina (radula)</i> | 4-sulfated on the galactose subunits (~25% ester sulfate) |
| <i>iota</i> - | <i>Eucheuma (spinosum)</i> | 4-sulfated on the galactose subunits and 2-sulfated on the 3,6-anhydrogalactose subunits (~32% ester sulfate) |
| <i>lambda</i> - | <i>Chondrus (crispus)</i> , <i>Gigartina (radula)</i> | Alternating 2-sulfated 1,3-linked α -D-galactose and 2,6-disulfated 1,4-linked β -D-galactose backbone (minimal 3,6-anhydro- β -D-galactose) (~35% ester sulfate) |

Table 2. Seaweed polysaccharides: important properties and selected applications

| Polysaccharides | Important properties | Selected applications |
|------------------------|---|--|
| Agars | Gel aqueous solutions at low concentrations Form thermoreversible gels Relatively inert Significant degree of hysteresis Retain moisture Resist hydrolysis by terrestrial microorganisms | Baking icings Jelly candies Canned meats Dental impression media Laxatives Microbial culture matrix Raw material for agarose |
| Agaroses | Gel aqueous solutions at low concentrations Form ion-dependent thermoreversible gels Controllable electroendosmosis (EEO) Minimal non-specific protein reactivity Significant degree of hysteresis | Matrices for: Electrophoresis Immunoassays Microbial and cell culture Chromatography Immobilized systems |
| Algins/alginates | Ammonium and alkali metal salts are soluble in water, whereas free alginic acid and alkaline earth and Group III salts are insoluble and can form gels Bind water Thicken aqueous systems Suspend solids | Frozen foods to maintain structure on thawing Baking icings Salad dressings Tabletting agent Dental impression media Textile sizing Matrices for immobilized systems |
| Carrageenans | Bind moisture Stabilize emulsions Control flow and texture properties of food systems High protein reactivity – strong interactions with milk proteins | Frozen dessert stabilizers Chocolate milk stabilizer Texturizers for low-fat foods Low-calorie jellies Toothpaste binders Air-freshener gels Personal care products Pet foods |
| <i>kappa</i> - | Form strong rigid aqueous gels with potassium and calcium ions Exhibit synergy with locust bean and konjac gums | |
| <i>iota</i> - | Form elastic aqueous gels with calcium ions Exhibit synergy with locust bean gum and starch Suspend particulates | |
| <i>lambda</i> - | Non-gelling aqueous system viscosifier | |

The development of analytical methods has been important in enabling the determination of molecular structures and classifying the polysaccharides produced by a given algal species, maintaining consistency of commercial polysaccharide products, and determining the fate of the polysaccharides in finished products. As appropriate analytical tools and techniques develop, they are being applied within the industry.

Significant variations in seaweed polysaccharide molecular structure can, and do, occur. The success of the industry has relied on both minimizing these variations for standardized products and exploiting them for new products.

Much of the above R&D has been supported within the marine algal polysaccharide industries and is surrounded by proprietary concerns. Pre-competitive base-building algal and algal polysaccharide research

has, however, been given only token support by the industry, and then only as it meets near-term needs.

Long-term R&D

Although genetic manipulation of land plants by recombinant DNA (rDNA) technology is becoming routine, little attention has been focused on applying this approach to marine macroalgae. Seaweeds are also amenable to genetic manipulation, through either conventional crossbreeding or the emerging techniques of molecular biology. However, many seaweed polysaccharides are destined for human consumption, and are therefore strictly regulated. In some countries, including the USA, only carrageenans from specific seaweeds meet food-ingredient regulations, and this discourages the quest for new species as sources of raw materials. Without question, regulatory hurdles will become more numerous or stringent and act as a

greater deterrent to industry's pursuit of 'engineered' seaweed polysaccharides for human consumption. For non-regulated applications, cost will determine product success.

In addition to their use in establishing a more accurate taxonomy of marine macroalgae, advances in molecular and cell biology techniques will facilitate:

- mutation and mutant selection;
- crossbreeding through protoplast fusion to create hybrids;
- reduction of marine macroalgae to single cells and their *in vitro* culture;
- use of seaweeds as production hosts for heterologous expression;
- identification of, and transfer of, algal biosynthetic pathways for polysaccharides or other desired materials into prokaryotic production hosts;
- metabolic engineering of algae to enhance the production of, for example, polysaccharides;
- identification of algal genetic control elements (e.g. promoters).

Appropriate vectors, or techniques, must be identified that can be used to introduce foreign genes. High efficiency transformation methods must be developed. Finally, the regeneration of sustained growth and/or reproduction-capable algae from transformed protoplasts and cells needs to be developed as a routine technique.

Taxonomy using molecular biology

Perhaps the biggest barrier to acceptance of new sources for the production of marine macroalgal polysaccharides in the current, strict regulatory environment is the need to prove equivalence of raw materials. Many regulations were established well before analytical methods such as NMR spectroscopy were available, and plant morphology was the main criterion in taxonomical classification of marine macroalgae. Because the species acceptable as sources have been identified by name in the regulations, alternative, perhaps more abundant or more easily cultivated, species are often excluded even though polysaccharide extracts from them may be essentially identical. Conversely, existing taxonomic classification has not been infallible, and some seaweeds classified as closely related actually contain different polysaccharides. Ultimately a combination of molecular biology, refined analytical methods and traditional morphological taxonomy will produce a more appropriate list of acceptable raw materials. A recent paper¹⁰ reporting on such an approach suggested a revision of systematics of one genera (Nizymeniaceae, Gigartinales, Rhodophyta) based on polysaccharide content, anatomy and nucleotide sequences.

The application of nucleic acid analysis to the taxonomy/algal classification problem has involved: (1) restriction enzyme fragment analysis; and (2) DNA sequence homologies. This research has enabled the creation of phylogenetic maps, and assisted the classification process.

However, anionic polysaccharides in marine macroalgae have similar properties to the nucleic acids (they are precipitated by ethanol at about the same pH as nucleic acids), and thus complicate isolation of the DNA and RNA. The presence of even minute quantities ($<1 \mu\text{g ml}^{-1}$) of these can interfere with restriction enzyme digestions, and has necessitated development of modified DNA and RNA isolation methods¹¹⁻¹³.

Electrophoresis of plastid DNA fragments, obtained by digestion with restriction endonucleases, has been used to determine relatedness in the red marine macroalgae¹⁴; one study¹⁵ used this technique to prove that two macroalgae previously thought to be distinct species were actually identical.

Targets for sequence-homology determination among the red seaweeds have been the 5S ribosomal RNA¹⁶, nuclear small-subunit rRNA genes¹⁷, the plastid *rbcL* gene coding for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo)¹⁸, nuclear genes encoding cytosolic and chloroplast glyceraldehyde-3-phosphate dehydrogenases (GADPH)¹⁹, gene amplification products²⁰, the β -tubulin gene²¹, subunit 3 of cytochrome *c* oxidase²², and small-subunit rRNA (SSU rRNA) from the mitochondria²³.

Nuclear DNA reassociation kinetics have also been used to determine inter- and intraspecific variations in selected agarophytes and carrageenophytes²⁴⁻²⁷.

Protoplast formation, callus culture and plant regeneration

At present, obtaining sufficient amounts of selected strains of marine macroalgae for cultivation remains a problem in that current methods require taking up to 25% of the cultivated material as the seed. This reduces productivity and, in cultivation efforts where the seedstock is attached to the supporting structure by hand, it is labor intensive. The ability to obtain viable macroalgal protoplasts that can divide, form cell walls and regenerate into whole plants is critical to a number of important processes, including the production of hybrid and transgenic macroalgae. If generation of viable protoplasts that form cell walls and divide could be achieved reproducibly and on a large scale, the protoplasts could be used as seedstock for macroalgal culture. Significant progress in this direction has been reported for one commercially important seaweed, *Porphyra linearis*²⁸. Success in obtaining viable protoplasts has been reported for *Gracilaria tikvahiae*²⁹, other strains of *Porphyra*³⁰, *Chondrus crispus*³¹ (followed by cell wall regeneration and division), *Gracilaria verrucosa*³² and *Kappaphycus alvarezii*³³. The paper on *K. alvarezii* also reports that the protoplasts secreted carrageenan fragments.

Progress has also been made in the field of callus, or undifferentiated cell, culture of marine macroalgae. Success has been reported for various *Gelidium* and *Gracilaria* agarophytes³⁴ and *Pterocladia capillacea*³⁵. Under appropriate conditions, seaweed polysaccharides can be produced by callus culture, including agar from *P. capillacea*³⁵. Whether this could ever be a

cost-effective production method for polysaccharides is doubtful. However, it is another way of providing cultivation seedstock and material for biosynthetic pathway determinations.

Gene mapping and sequencing

A prerequisite for either genetic or metabolic 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 microorganisms have been used with success to locate specific genes. Considerable groundwork has been done using unicellular red and green algae, and cyanobacteria, but reports of mapping and sequencing red marine macroalgal genomes are sparse. The chloroplast ribosomal-protein-encoding genes of the agarophyte *Gracilaria tenuistipitata* have been located, cloned and characterized³⁶. 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 rp122 protein in *G. tenuistipitata* has also been isolated and sequenced³⁷.

The polymerase chain reaction (PCR) works with seaweed DNA and has been used to amplify *Gracilaria pacifica* nuclear and plastid ribosomal genes from algal herbarium specimens and algal spores³⁸. The glyceraldehyde-3-phosphate dehydrogenase (GADPH) gene system of the red alga *C. crispus* has been investigated³⁹. Promoter structures, intron/exon organization, genomic complexity, differential expression of genes and transcript level of the genes in gametophytes and protoplasts were determined, paving the way for genetic transformations in *Chondrus*.

Transformation, protoplast fusion

Methods for the introduction of foreign genes into other organisms include protoplast fusion; introduction of the desired gene(s) by appropriate vectors, including insertion of transformed plasmids; infection with transformed viruses or other specific pathogens; and direct insertion of the gene by, for example, electroporation or ballistics.

In order to use the transformed plasmid technique, plasmids must be present in the host. Five of 21 red algal genera studied⁴⁰ were found to contain circular dsDNA plasmids. Some of these were isolated and characterized, and one 3.5 kbp plasmid from *Gracilaria lemaneiformis* was sequenced to reveal two potential open reading frames. In this species, plasmids are present in a high copy-number per 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 determined⁴¹. This 3827 bp circular plasmid has one major open reading frame that generates a transcript and could encode a 411 amino acid polypeptide.

In attempting to determine whether protoplast fusion techniques could be used for algal transformation, factors affecting the electrofusion efficiency of

Porphyra yezoensis protoplasts were studied⁴². Fusion efficiencies were found to depend on the concentration and nature of reagents used to adjust the osmotic pressure of the medium. A recent paper⁴³ suggests that recombinant viruses, particularly the large dsDNA viruses that are known to infect eukaryotic algae, can be used as transformation vectors for marine macroalgae.

Impact of current research on commercial concerns

To sustain growth in a competitive environment, the seaweed polysaccharide industries devote significant effort to reducing production costs and to finding new applications for polysaccharides. Most research supported by industry, both internally and externally, is therefore focused on short-term needs. However, ongoing basic-research efforts to classify marine macroalgae by the chemical structure of the polysaccharides they produce and their relatedness as determined by genomes, and to develop new strains and improve seaweed culture methods, are also directly relevant to industry. The first of these, in particular, is an important step towards gaining regulatory acceptance of a wider range of source species.

Long-range research into elucidating the biosynthetic pathways for polysaccharide(s) production, and the development of methods for genetic manipulation, are prerequisites to engineering macroalgae capable of synthesizing, for instance, polysaccharides enzymatically processed by the algae so that only aqueous extraction and recovery or washing and grinding are needed to prepare commercially acceptable products. Such technological advances are also essential for the development of seaweeds as heterologous production hosts. If mammalian antibodies can be produced in tobacco plants⁴⁴ and the rabies virus glycoprotein in tomatoes⁴⁵, why not value-added products in seaweeds? Macroalgae may be a cheaper alternative 'host' to produce these products.

Overcoming existing barriers

Many, but not all, of the barriers to acceptance of new marine macroalgal raw material and new or altered polysaccharides are regulatory. Probably the single most important advance would be to base regulations on analytical data for polysaccharide(s) that are present rather than rely on the outmoded classical seaweed taxonomy. Not only would this open up new supply channels for basic raw materials for industry, but by doing so would create economic opportunities in some developing countries.

Further into the future, products from genetically altered algae need to be considered: they will present the same regulatory problems that surround genetically engineered land plants whose products are targeted for human consumption. The main consideration for polysaccharides from these algae will probably, however, be whether they offer a cost-effective advantage. A parallel situation may be plants such as maize or potatoes engineered for altered starch

composition. There may be sufficient applications for engineered polysaccharides in the non-consumable markets to warrant the cost of producing them.

Pre-competitive research is slowed by the low level of funding. Inevitably, researchers focus on areas with the most abundant support, and more funding is needed if the field is to advance at a rate more in line with its potential importance.

Future prospects

Polysaccharides from marine macroalgae are the basis of well-established, growing industries. Many consumer products rely on their unique properties and would not exist without their availability. Important advances in modern medicine and biotechnology would not have been possible without their existence^{46,47}. With increased research emphasis, new applications and possibly even new seaweed polysaccharides will emerge. Genetic understanding and techniques for the insertion of foreign genes are evolving. Overall, the future looks bright for marine macroalgae and the polysaccharides they produce. However, to sustain continued growth, the industries involved must support scientists working to develop the pre-competitive research base upon which future, commercially important discoveries will be made.

References

- Zilinskas, R. A. and Lundin, C. G. (1993) *Marine Biotechnology and Developing Countries* (World Bank Discussion Paper, No. 210), p. 29, The World Bank
- Bird, K. T. (1995) *Sea Technol.* April, 58–62
- Arminsen, R. and Galatas, F. (1987) in *Production and Utilization of Products from Commercial Seaweeds* (McHugh, D. J., ed.), pp. 1–57 (FAO Fisheries Technical Paper, 288)
- Selby, H. H. and Whistler, R. L. (1993) in *Industrial Gums: Polysaccharides and their Derivatives* (3rd edn) (Whistler, R. L. and BeMiller, J. N., eds), pp. 87–103, Academic Press
- Renn, D. W. (1984) *I&EC Product R&D* 23, 17–21
- McHugh, D. J. (1987) in *Production and Utilization of Products from Commercial Seaweeds* (McHugh, D. J., ed.), pp. 58–115 (FAO Fisheries Technical Paper, 288)
- Clare, K. (1993) in *Industrial Gums: Polysaccharides and their Derivatives* (3rd edn) (Whistler, R. L. and BeMiller, J. N., eds), pp. 105–143, Academic Press
- Stanley, N. F. (1987) in *Production and Utilization of Products from Commercial Seaweeds* (McHugh, D. J., ed.), pp. 116–146 (FAO Fisheries Technical Paper, 288)
- Therkelsen, G. H. (1993) in *Industrial Gums: Polysaccharides and their Derivatives* (3rd edn) (Whistler, R. L. and BeMiller, J. N., eds), pp. 145–180, Academic Press
- Chiiovitti, A., Kraft, G. T., Saunders, G. W., Liao, M.-L. and Bacic, A. (1995) *J. Phycol.* 31, 153–166
- Su, X. and Gibor, A. (1988) *Anal. Biochem.* 174, 650–657
- Roell, M. K. and Morse, D. E. (1991) *J. Phycol.* 27, 299–305
- Saunders, G. W. (1993) *J. Phycol.* 29, 251–254
- Goff, L. J. and Coleman, A. W. (1988) *J. Phycol.* 24, 357–368
- Bird, C. J., Nelson, W. A., Rice, E. L., Ryan, K. G. and Villemur, R. (1990) *J. Appl. Phycol.* 2, 375–382
- Van den Eynde, H., De Baere, R. and De Wachter, R. (1987) *Arch. Int. Physiol. Biochem.* 95, B244
- Ragan, M. A., Bird, C. J., Rice, E. L., Gutell, R. R., Murphy, C. A. and Singh, R. K. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 7276–7280
- Freshwater, D. W., Fredericq, S., Butler, B. S., Hommersand, M. H. and Chase, M. W. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 7281–7285
- Liaud, M.-F., Valentin, C., Martin, W., Bouget, F.-Y., Kloareg, B. and Cerff, R. (1994) *J. Mol. Evol.* 38, 319–327
- Saunders, G. W. and Kraft, G. T. (1993) *Can. J. Bot.* 72, 1250–1263
- Liaud, M.-F., Brandt, U. and Cerff, R. (1995) *Plant Mol. Biol.* 28, 313–325
- Boyen, C., Leblanc, C., Bonnard, G., Grienenberger, J.-M. and Kloareg, B. (1995) *Nucleic Acids Res.* 22, 1400–1403
- Leblanc, C., Kloareg, B., Loiseaux-der Goer, S. and Boyen, C. (1995) *J. Mol. Evol.* 41(2), 196–202
- Dutcher, J. A., Kapraun, D. F. and Sizemore, R. K. (1990) *J. Appl. Phycol.* 2, 259–267
- Kapraun, D. F., Dutcher, J. A. and Lopez-Bautista, J. (1992) *J. Appl. Phycol.* 4, 129–137
- Kapraun, D. F., Dutcher, J. A., Bird, K. T. and Capecchi, X. (1993) *J. Appl. Phycol.* 5, 99–107
- Kapraun, D. F., Bailey, J. C. and Dutcher, J. A. (1994) *J. Appl. Phycol.* 6, 7–12
- Chen, L. C.-M., Craigie, J. S. and Xie, Z. K. (1994) *J. Appl. Phycol.* 6, 35–39
- Cheney, D. P. (1984) in *Biotechnology in the Marine Sciences* (Colwell, R. R., Pariser, E. R. and Sinskey, A. J., eds), pp. 161–175, Wiley & Sons
- Polne-Fuller, M. and Gibor, A. (1986) *Aquaculture* 57, 117–123
- Le Gall, Y., Braud, J. P. and Kloareg, B. (1990) *Plant Cell Rep.* 8, 582–585
- Bellanger, F., Verdus, M. C., Henocq, V. and Christiaen, D. (1990) *Hydrobiologia* 204/205, 527–531
- Zablackis, E., Vreeland, V. and Kloareg, B. (1993) *J. Exp. Bot.* 44, 1515–1522
- Gusev, M. V., Tambiev, A. H., Kirikova, N. N., Shelyastina, N. N. and Aslanyan, R. R. (1987) *Mar. Biol.* 95, 593–597
- Liu, X.-W., Rochas, C. and Kloareg, B. (1990) *J. Appl. Phycol.* 2, 297–303
- Kao, J.-s., Wu, M. and Chiang, Y.-M. (1990) *Gene* 90, 221–226
- Kao, J.-s. and Wu, M. (1990) *Nucleic Acids Res.* 18, 3067
- Goff, L. J. and Moon, D. A. (1993) *J. Phycol.* 29, 381–384
- Liaud, M.-F., Valentin, C., Brandt, U., Bouget, F.-Y., Kloareg, B. and Cerff, R. (1993) *Plant Mol. Biol.* 23, 981–994
- Goff, L. J. and Coleman, A. W. (1990) *Curr. Genet.* 18, 557–565
- Villemur, R. (1990) *Plant Mol. Biol.* 15, 237–243
- Mizukami, Y., Kito, H. and Okauchi, M. (1993) *J. Appl. Phycol.* 5, 29–36
- Henry, E. C. and Meints, R. H. (1994) *J. Appl. Phycol.* 6, 247–253
- Ma, J. K.-C. and Hein, M. B. (1995) *Trends Biotechnol.* 13, 522–527
- McGarvey, P. B. et al. (1995) *Biotechnology* 13, 1484–1487
- Renn, D. W. (1990) *Hydrobiologia* 204/205, 7–13
- Renn, D. W. (1992) in *Marine Biotechnology: Pharmaceutical and Bioactive Natural Products* (Vol. 1) (Attaway, D. H. and Zaborsky, O. R., eds), pp. 181–196, Plenum Press

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