

ALGAL



BIOTECHNOLOGY

Edited by
T. STADLER, J. MOLLION,
M.-C. VERDUS, Y. KARAMANOS,
H. MORVAN and D. CHRISTIAEN

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T. STADLER, J. MOLLION, M.-C. VERDUS,
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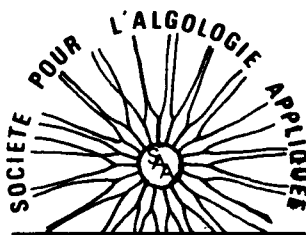
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P R E F A C E

Biotechnology research development foresees, in the near future, a "revolution" in algal product exploitation : tissue culture, genetic manipulations, energy, nutrition, etc... Arne JENSEN (1987), in his opening address to the 12th International Seaweed Symposium, underlined that much was still unknown about the development of algae as compared to that of cultivated terrestrial plants. In fact, with a few exceptions like *Eucheuma* in the Philippines, cultivation techniques and algal selection are still trying to find their way. Moreover, in plants of economical interest, some exploitation difficulties linked to natural variations have been removed owing to molecular biology and cell biotechnology progress.

Given these circumstances, the **Société pour l'Algologie Appliquée** and the **Equipe Polysaccharides Pariétaux des Végétaux** proposed that scientists dealing with algal biotechnology get together to bring forth recent accomplishments in their fields. The relevance and opportunity of this theme afforded the gathering of 170 industrial and scientific people from 18 countries. The topics of the meeting were presented through plenary sessions, 42 contributed papers and 3 round tables.

Reading the articles collected in this work presents tissue culture and protoplast preparation as the techniques for the future. They sometimes are necessary to remove obstacles due to the complexity of algal life cycles, and to allow selection and hybridization for the regeneration of new plants.

The economic feasibility of these new culture techniques, developed at the research level, is, however, bound by the development of new bioreactors. You will find in this text the most up-to-date results of concepts and realizations of pilot-scale cultivation systems destined to industrial production.

Polysaccharides constituted the third major theme of the meeting. A round table, dedicated to the memory of Professor W. YAPHE, dealt, in a lively manner, with the industrial future of phycocolloids. Despite improvements in

production conditions, microalgal polysaccharides are still trying to find their way in the market of gelling products.

The research effort appears to be essentially carried out at the cellular level (protoplast production). The following step is taking shape at the molecular level in search of metabolic enzymes to improve the quality and properties of phycocolloids.

One can expect decisive progress in this field and the meeting in 1990, in Israel, promises to be very interesting.

The Editors

INTRODUCTION

Le développement de la Recherche en matière de Biotechnologies rend prévisible à court terme une "révolution" dans l'exploitation des produits des algues : culture de tissus, manipulations génétiques, énergie, alimentation, etc... Arne JENSEN (1987), dans son discours d'introduction au 12th International Seaweed Symposium, soulignait que le développement des Algues était encore loin de celui des Plantes terrestres cultivées. En effet, mis à part quelques rares exemples, comme aux Philippines avec *Eucheuma*, les pratiques culturales et la sélection cherchent encore leur voie. De plus, chez les Végétaux d'intérêt économique, un certain nombre de difficultés d'exploitation liées aux variations naturelles ont pu être levées grâce aux progrès de la Biologie moléculaire et des Biotechnologies cellulaires.

C'est dans ce contexte que la Société pour l'Algologie Appliquée et l'Equipe Polysaccharides Pariétaux des Végétaux ont proposé aux Scientifiques concernés par la Biotechnologie des Algues de faire le point des récents

développements de leurs spécialités. La pertinence et l'opportunité du thème a permis de réunir 170 personnalités industrielles et scientifiques de 18 pays autour de 42 communications, introduites par des conférences plénières et reprises par 3 tables rondes.

La lecture des articles réunis dans cet ouvrage révèle que la culture de tissus et la préparation de protoplastes s'imposent comme des techniques d'avenir. Elles représentent parfois un passage obligé pour lever les obstacles dus à la complexité des cycles biologiques des Algues et permettre la sélection ou l'hybridation en vue de la régénération de nouveaux plants.

Mais la viabilité économique des nouveaux procédés de culture mis au point par la Recherche est liée aux développements de nouveaux bioréacteurs. Vous trouverez dans ce livre les derniers résultats de conceptions et de réalisations de pilotes de culture destinés à la production industrielle.

Les polysaccharides constituent le troisième volet majeur du Congrès. Une table ronde, dédiée à la mémoire du Professeur W. YAPHE, a abordé de façon animée l'avenir industriel des phycocolloïdes. Malgré l'amélioration des conditions de production, les polysaccharides des microalgues se disputent toujours une place sur le marché des gélifiants.

L'effort de Recherche est donc essentiellement porté au niveau cellulaire (fabrication de protoplastes). L'étape suivante se dessine et se situe au niveau moléculaire dans la recherche des enzymes métaboliques des phycocolloïdes de manière à améliorer leurs qualités et leurs propriétés.

On peut s'attendre à des progrès importants en la matière et le rendez-vous de 1990 en Israël promet d'être très intéressant.

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THE DEVELOPMENT AND GENETIC IMPROVEMENT OF MARINE CROPS

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ABSTRACT

An important step in the domestication of a wild species is the acquisition of human control over its reproduction. This control requires knowledge of the plant's life cycle, and of the important environmental factors affecting reproduction. Once this information is available, selective breeding can begin. Plants under human management are not subject to the same constraints as in nature, thus extensive changes to the original genotype are possible. Even mutations normally deleterious to survival can be incorporated into cultivated strains to improve properties desired by growers. The domestication of marine plants has lagged far behind that of terrestrial crops; however, twentieth-century plant breeding and genetics, including the new techniques of molecular genetics, should allow rapid progress once the commitment to grow a species is made. Genetic progress for existing marine crops is summarized.

INTRODUCTION

We shall never know how mankind was introduced to marine plants. Perhaps seaweeds were already occasional items in the diet of pre-human homonids. Perhaps the first human to eat seaweed was a desperate, starving woman trying to feed her young. Perhaps it was prepared for a tribal chieftain seeking something new for a jaded palate. We can only

speculate. What is certain is that somewhere, long ago, humans found the taste of certain seaweeds acceptable, and added them to their diet.

Although it was not a dramatic event, the realization that seaweeds have food value was the first small step towards the eventual development of marine crops. Early events in seaweed domestication probably paralleled the development of terrestrial crops in that a process of trial and error was needed to learn about the characteristics of various plants. Harvesting of certain wild algae became part of the activity of coastal communities. How to recognize the best species, and where and when to find them became knowledge passed from one generation to the next.

Cultivation of marine crops has lagged far behind the domestication of terrestrial plants, probably because seaweeds were much less important in the diet than staples provided by terrestrial crops. The reproduction of marine plants was also more difficult to understand, and the marine environment more difficult to manage. Nevertheless, the high demand for certain seaweeds, particularly Porphyra, led some people to place sticks and stones into the water to try to get 'seed' which could then be tended in a protected location [1]. Although not very reliable, this activity marked the beginning of the seaweed cultivation industry. The practice of capturing wild 'seed' of Porphyra continued until just a few decades ago, which emphasizes the recentness of seaweed domestication .

Phycological studies during the past century have provided the critical information on life histories needed to initiate improvement of marine crops. With knowledge of algal life cycles, it became possible for the first time to control, or at least predict, the timing of reproduction, which is probably the most important advance for the domestication of any species. Control of reproduction permits the scheduling of specific steps in the crop production cycle, and permits selection of superior plants as "seed stock" for the next crop. Even when genetic understanding is minimal, some crop improvement can be

achieved by careful selection of breeding stock from previous crop generations and wild populations. With the advent of such selection, genetic improvement of the crop is initiated, and the stage is set for more scientific application of genetic principles.

The pace of genetic improvement of marine crops has not been dramatic, but there is a broad research front that has been contributing information on plant breeding, formal genetics and molecular genetics. The most directly applied of these is, of course, selective breeding, which is little different from plant breeding of terrestrial crops, though the monoplloid condition of many algae greatly simplifies the experimental designs. Formal genetic studies of individual genes, sometimes called Mendelian analysis, provides well-characterized marker genes useful in a variety of applications. They also provide new mutations that might be useful for incorporation into a breeding program. Molecular genetics, with its ability to manipulate DNA directly, will contribute to crop improvement in the future, but at present remains more promise than real progress. There is still much to learn before the many technical blocks to genetic engineering of marine plants are removed. Nevertheless, there is an increasing amount of information on algal genes and genomes. Although this kind of information is not immediately useful for crop improvement, it provides a body of fundamental knowledge that will benefit future generations of breeders, and may lead to some biotechnological innovations even before genetic engineering of the plants is feasible. In this presentation I will set most speculation aside and concentrate on current genetic progress towards the development of existing marine crops.

CURRENT APPLICATION OF GENETICS TO MARINE CROPS

Porphyra

Although the harvesting and utilization of Porphyra are deeply rooted in Oriental cultures [1], it was not until comparatively

recently that it became a highly successful, economically important marine crop. The key to its development was a basic phycological discovery, about 40 years ago, that the shell-boring genus Conchocelis was actually the sporophyte phase of Porphyra [2]. This discovery made it possible to control the life cycle of Porphyra and to manipulate it in culture.

The industry has already benefitted from genetic improvement of cultivated varieties. Strain selection has been underway for about a decade in Japan, although little of this information has appeared in published literature. One of the early breeding targets was larger fronds, and significant improvement over the wild-type strains has been reported [3]. The increased frond length appears to be partially due to delayed fertility, which allows a longer growing period before the fronds begin to erode by release of gametes and spores. Selection has also been used to obtain strains adapted to local growing conditions. At the present time Porphyra growers have a large number of species and strains available for use in various localities. In Japan alone there are more than 30 recognized cultivars, most of them derived from P. yezoensis and P. tenera, and probably hundreds of additional strains selected informally by individual growers [T.F. Mumford Jr. pers. comm.]. Strain selection in the coming years will likely place emphasis on efficiency and quality rather than quantity, since overproduction has been a problem for the industry. Disease, whether due to pathogens or physiological stress, will continue to be a problem for the industry, and it is reasonable to assume that genetic resistance will be sought. More monospore-producing strains may be selected since monospores can compensate for a poor conchospore seeding of nets or even substitute for conchospores entirely [4].

Formal genetic studies have not yet contributed very much to the commercial aspects of the Porphyra industry. Information obtained from fundamental genetic studies is still very rudimentary, being limited to the Mendelian analysis of a few color mutations of Porphyra yezoensis [5-7]. Nevertheless, a very important discovery has emerged. In crosses between color mutants it was discovered that gametophytes derived from a heterozygous conchocelis were usually mosaics. The

fronds of these plants were divided into two or more sectors having different colors. It was soon realized that sectored plants originated from meiosis, which was completed in the germinating conchospore [8], and not in the conchosporangium as had been thought. Similar observations on the timing of meiosis have now been made in other species of Porphyra [9; Mitman and van der Meer, unpublished results] which suggests that it may be a general feature of the sexual life history. Clearly this fundamental discovery has implications for Porphyra breeders. They must recognize that every plant of Porphyra is potentially a genetic mosaic with up to four different genotypes in different portions of the frond. Simply growing a plant in isolation to force self-fertilization is not sufficient to guarantee that the offspring will be uniformly inbred.

A substantial amount of molecular genetic information will be available for Porphyra in the near future. Characterization of the Porphyra genomes is already in progress, and the first information on the chloroplast genome has been presented at a recent conference [10]. There has been more progress in the manipulation of cells and protoplasts of Porphyra than of other red algae [11, 12], making it possible that the first transgenic red algal plants will be formed in this genus. The precise role such molecular genetic studies will play in the improvement of Porphyra strains is not yet clear, but the transfer of specific single gene characteristics between species would be an obvious application.

Laminaria

Chinese scientists have led the world in the genetic development of highly productive lines of the brown seaweed Laminaria japonica, which is now extensively cultivated in China [1]. Starting with a small harvest in the mid-1950's, Laminaria cultivation has steadily increased to the point where about 250,000 dry tonnes are harvested annually. Half of the harvest is consumed as food, Laminaria being an important source of iodine in the Chinese diet. The other half goes to

industry, principally for the extraction of alginate and mannitol.

Plant-breeding studies conducted during the 1960's demonstrated that the commercially important morphological characteristics and iodine content of Laminaria were under quantitative genetic control [13]. Soon after, a program of selection and inbreeding was undertaken to obtain and stabilize superior lines for aquaculture [14]. During the 1970's a novel experimental approach for the breeding of Laminaria was developed based on the observation that sporophytes can develop by parthenogenesis [15]. Such parthenosporophytes start as haploids but many become fertile, homozygous diploids by spontaneous chromosome doubling early in development. These diploids can be used to help evaluate the sporophytic characteristics of individual genomes, which cannot be done with heterozygous diploids obtained in the normal cycle. A female identified as having a superior genotype in such evaluations, was used to obtain an improved commercial line [16]. Most recently, normal female gametophytes were hybridized with a male plant from Japan to obtain what is described as the most luxuriantly growing, high-iodine line achieved thus far [17]. Claims that the vigor of the hybrid comes from heterosis remain to be substantiated in properly designed experiments.

Apart from the Chinese work, few genetic studies have been reported for Laminaria. Some hybridizations have been made in pursuit of taxonomic or ecological questions [18-20], and one brief study reported on the heritability of alginate content [21]. Other species of brown algae are almost unstudied; however, there is steadily increasing cultivation of Undaria pinnatifida, which may be accompanied by unreported strain selection. A decade ago there was expressed interest in the use of interspecific hybrids of giant kelps for aquaculture [22, 23]; however, this now appears to be only of historical interest.

Formal genetic studies of Laminaria and other brown algae essentially do not exist. One mutation, which causes brown spots on the blades of L. japonica has been described and partially characterized [24]. This mutant, and the observation that sex determination in the kelps is genetically determined, appears to be the