Domestication of indigenous Porphyra (nori) species for commercial cultivation in Northeast America

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Domestication of indigenous *Porphyra* (nori) species for commercial cultivation in Northeast America

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The red alga *Porphyra* (commonly called nori), is a major source of food for humans throughout the world and is the most valuable maricultured seaweed in the world today. In 1995, approximately 12 billion sheets (approximately 36,000 metric tons) were produced, with an annual value of more than $1.8 billion. *Porphyra* is primarily used as the reddish-black wrapping, consisting of chopped, pressed, and toasted blades, around sushi rolls. Nori also is a major source of taurine, which controls blood cholesterol levels, and it is a staple in macrobiotic diets. Nori contains high levels of proteins (25-50 percent), vitamins (higher vitamin C content than in oranges), trace minerals and dietary fiber.[6,9] It also serves as a preferred source of the red pigment r-phycoerythrin, which is utilized as a fluorescent tag in the medical diagnostic industry. The U.S. is primarily dependent upon Japan and to a lesser extent China and Korea, for imports of nori.

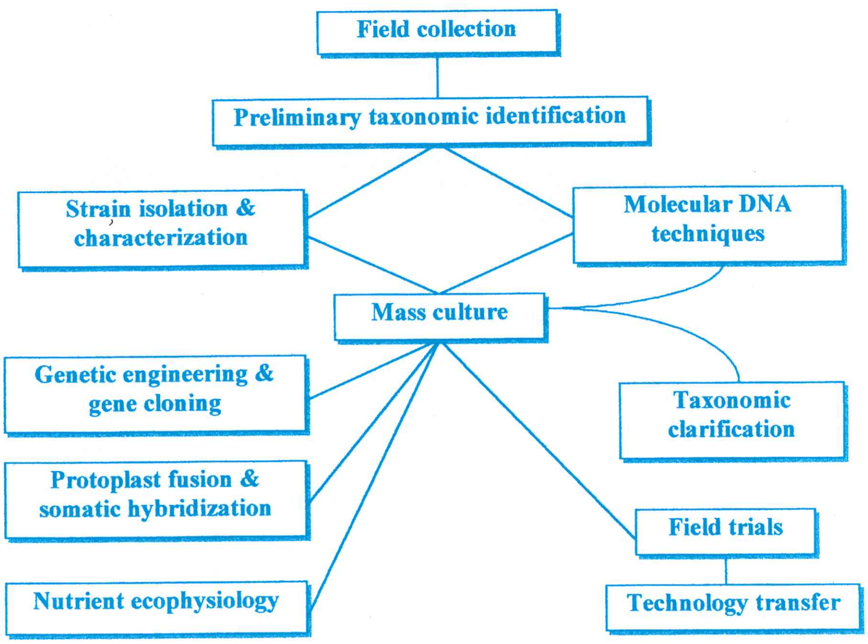

Fig. 1. Domestication process for the development of a commercial Porphyra *strain.*

Previous attempts to culture nori on the West Coast of the United States and Canada have been unsuccessful. The failure was not due to the market size, economic viability of the participants, or the biological aspects of cultivation, but solely due to the inability of nori farmers to obtain aquaculture lease permits in the coastal waters of Washington State. Political pressure brought to bear by landowners and commercial fisherman was too much for the fledgling industry to overcome.[7] The political forces that resulted in the collapse of the Washington State effort have not been present in coastal New England. In initiating a nori cultivation program in northeastern Maine (i.e. Cobscook Bay, Washington County), Coastal Plantations International, Inc. (CPI), received support of local, state and federal agencies, as well as a popular interest. The development of a labor-intensive seaweed aquaculture industry is expected to significantly reduce the unemployment rate and the reliance on a single dominant but vulnerable, source of employment- salmon-farming. The State Legislatures in Maine, Connecticut and other New England States have been overwhelmingly supportive of lease site acquisition, stature changes and extension support.

Given the lack of understanding of the biology of the native New England nori species, CPI. has primarily utilized a commercially valuable Asiatic taxon, *Porphyra yezoensis*, which was developed during the 1960s by strain improvement programs on *P. tenera* and *P. yezoensis*. Although *P. yezoensis* has many desirable features, it had been selected for growth conditions in northern Japan and is having serious difficulty in dealing with northeastern Maine's coastal environment. Therefore, it was logical to establish a cultivar improvement program for local *Porphyra* species, just as has been done in Japan. Through such a program, genetically improved nori cultivars which should be better adapted to local conditions could be developed.

In 1995, the National Sea Grant College Program, NOAA, Department of Commerce (in cooperation with several New England Sea Grant College Programs including those in the States of Connecticut, Maine, New Hampshire and Massachusetts) awarded a regional grant to promote the domestication of indigenous species of *Porphyra* for commercial cultivation (Fig. 1). Nori cultivation has the single greatest potential for generating a viable seaweed aquaculture industry in the United States. Whether or not seaweed aquaculture will ultimately succeed in the Northeast will depend in large part upon several key factors, including: (1) successful transfer and modification of Japanese and Chinese nori cultivation technology; (2) development of genetically improved species (cultivars) of marketable nori that will extend the growing and harvest season in New England; (3) establishing a constant supply of a seedstock of juvenile organisms that will be readily available; and (4) expansion of the area presently used for cultivation (i.e. beyond Cobscook Bay in Washington County).

Indigenous nori species in Northeastern America

In a recent synopsis of red algae from the Canadian Maritime Provinces, Bird and McLachlan record six species of *Porphyra* (i.e., *Porphyra amplissima* (Kjellman) Setchell and Hus in Hus, *P. miniata* (C. Agardh) C. Agardh, *P. umbilicalis* (Linnaeus) J. Agardh, P. *linearis* Greville, *P. purpurea* (Roth) C. Agardh, and *P. leucosticta* Thuret in Le Jolis).[3] A similar situation exists in New England, where five of these same taxa are recorded as well as others such as *P. carolinensis* Coll et Cox and *P. dioca* Brodie *et* Irvine. There may be an underestimation of species richness for this geography and a detailed taxonomic revision is required.

Species composition and distribution of *Porphyra* in New England and the Canadian Maritimes

To help clarify the taxonomic status and ecological requirements of *Porphyra* species in the Northeast, detailed seasonal and spatial collections are being made. A series of parameters, including depth, exposure, salinity, substratum type, etc. are recorded at each collection site. Morphometric data (i.e. length, width, color etc.) is measured for each individual sampled. Initially, 10 to 15 representative populations of each species have been sampled over the geographical range of the study and additional populations (10 to 20) have been sampled at locations determined by the results of the initial sampling. Molecular DNA techniques are being employed for genetic evaluations. More than 130 unialgal cultures of *Porphyra* have been established from Long Island Sound to the Canadian Maritimes.

These collections have shown that *P. umbilicalis* is by far the most abundant species spatially and temporally within the Gulf of Maine and Long Island Sound. It occurs throughout the year within diverse coastal and estuarine habitats, i.e. in the mid to low intertidal zones in the Gulf of Maine and into the upper intertidal in Long Island Sound. *P. amplissima* is most abundant within the northern Gulf of Maine, particularly occurring during

Fig. 2. Porphyra leucosticta *growing epiphytically in the upper subtidal. (Photo by Robert Wilkes)*

the spring-summer, within the low intertidal and subtidal zones and appears to be absent south of New Hampshire. *P. linearis* is presently forming localized ephemeral populations within the upper intertidal zones of open coastal habitats. It occurs within the Gulf of Maine in the winter and disappears by early spring. In eastern Long Island Sound it occurs in January and disappears in late March but is rarely found in western Long Island Sound. *P. leucosticta* fronds are initiated in early winter on the fronds of *Dumontia contorta* and *Chondrus crispus* within open coastal tide-pool habitats from the Gulf of Maine to Long Island Sound. As the winter progresses, it may be found epiphytically on other algae in the mid to low intertidal zones and into the upper subtidal zones and by early summer the leafy thalli are only present subtidally (Fig. 2). Limited knowledge is available about the phenology of *P. purpurea*. It is enigmatic in its distribution, with initial reports only occurring in the Canadian Maritimes. However, a form species now appears to be common as far south as Long Island Sound occurring throughout the summer. There appears to be distinct genetic differences between northern and southern populations. A species commonly confused with *P. purpurea, P. dioca*[4] is also found in Long Island Sound throughout the summer. *P. carolinensis* appears in Long Island Sound from late Fall to Winter growing epiphytically on *Fucus vesiculosus*.

Establishment of unialgal cultures of the different *Porphyra* taxa and strains

Cultures have been initiated from the native taxa described above. Small portions of blades are scrubbed with cotton swabs in sterile seawater. Tissue is then dried at 10°C for 24 hours and then reimmersed in an enriched seawater culture medium (von Stosch's or Provasoli's Enriched Seawater). After spore discharge (anywhere from 1 to 24 hours), the sections are saved for future genetic analyses. Individual carpospores (or monospores) are cultured and the resulting vegetative conchocelis stages (Fig. 3) (or regenerative monospore producing plantlets) are then maintained and cultured at 5-20°C, 10-50?mol photon m-2s-1, under different photoperiods (16:8; 12:12; Light:Dark). The extensive conchocelis and vegetative-thallus culture collection of *Porphyra* (both native and non-native species) that has been established is being used in a number of experiments designed to determine the optimum conditions for growth and reproduction of the native species (Table 1).

Chromosome studies

A variety of different cytological methods can be used in determining the number of chromosomes in a species. This method has proven useful in helping to delineate the different form-species found among *Porphyra* populations. *P. leucosticta*, for example, is found in populations at both Westport, MA, USA and Sandy Cove West, Nova Scotia, Canada. The Massachusetts population has a chromosome number of N=2 whereas the Nova Scotian population is found with N=3. Other reports have also identified *P. leucosticta* with N=3.[5] A similar population divergence is apparent with P. purpurea. Samples taken from Nova Scotia at Herring Cove and Avonport have a chromosome number N=5.[8] However, in Dipper Harbour and Grand Manan Island, New Brunswick Canada P. purpurea is found with N=2. This suggests that *P. purpurea* is represented by two distinct genetic entities or form-species.

Comparison of the genetic structure of *Porphyra* species and populations

A major step in the domestication process is the identification of strains with highly heritable, advantageous characteristics. Only after such traits are identified can the next phase of domestication and exploitation, the breeding or genetic manipulation of strains for specific characteristics, take place. In much of terrestrial agriculture this phase of development occurred hundreds of years ago, although such efforts still continue. An extensive selection and breeding program for Chinese seaweeds that were previously introduced from Japan has already been conducted. An attempt was made to generate new strains with higher temperature tolerances and increased iodine contents, although only the latter experiments were successful.

For each population sampled in the seasonal collections of the present project molecular DNA analyses have been undertaken alongside the traditional systematic analyses and culture studies. For each sequence class represented in our survey of Western North Atlantic *Porphyras*, we are using the Blast algorithm (Altschul *et al*, 1990) and the 18S rRNA gene to identify the most closely related DNA sequences of our *Porphyra* collections to those in Genbank, a worldwide database of DNA sequences. We have made tentative morphological identifications of more than 750 individuals and matched them to other *Porphyra* 18s sequences. Among all Genbank *Porphyra* sequences, the range of between-sequence divergences is 0.5 percent to 15 percent (i.e. between 85 to 99.5 percent similarity). There is no absolute criteria for assigning a percentage sequence divergence to between or within-species variation, however, sequence divergences of <1 percent probably represent within-species variation. Using these molecular techniques, we have found sequence divergences of more than 7.5 percent and this has enabled us to successfully discriminate between and among species of *P. purpurea, P. umbilicalis, P. leucosticta, P. carolinensis, P. dioca and P. linearis*. The integration of traditional systematic and new molecular techniques is now enabling us to re-evaluate the taxonomic status of *Porphyra* species throughout Northeast America.

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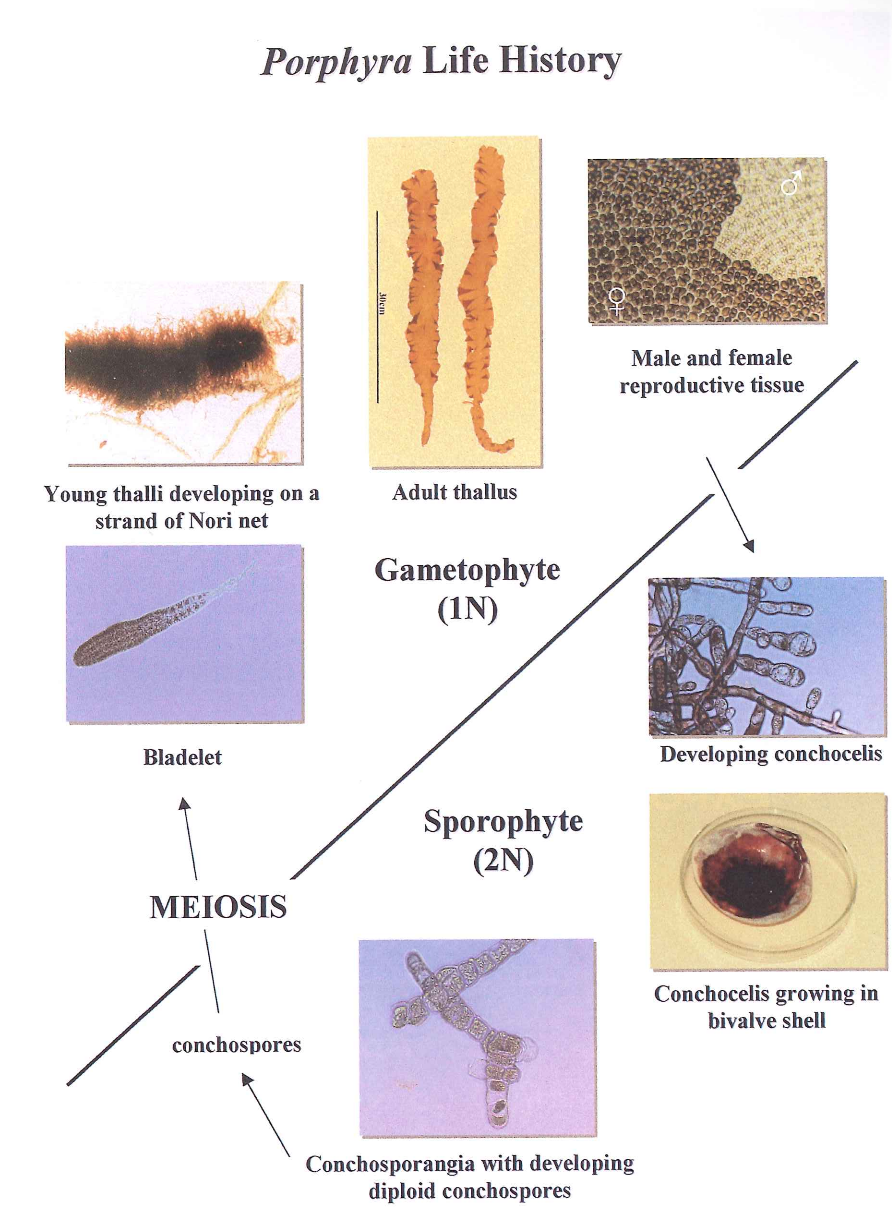

Fig. 3. Porphyra *life history.*

Table 1. Physiological parameters for *Porphyra* species from New England and the Canadian Maritimes

Species	Conditions for vegetative conchocelis growth	Conditions for conchospore maturation	Conditions for leafy thallus maturation
P. amplissima	5-100µmol photons m^{-2} s^{-1} @ 5-15°C	2-4 weeks @5-25µmol photons m^{-2} s^{-1}, 5-15°C	~4 weeks @ 50-100µmol photons m^{-2} s^{-1}, 5-15°C
P. linearis	5-100µmol photons m^{-2} s^{-1} @ 5-10°C	4-6 weeks @ 10-40µmol photons m^{-2} s^{-1}, 5-10°C	
P. leucosticta	5-100µmol photons m^{-2} s^{-1} @ 5-15°C	~4 weeks @ 10-40µmol photons m^{-2} s^{-1}, 10-15°C	
P. umbilicalis	5-100µmol photons m^{-2} s^{-1} 5-15°C		(monospores)10-100µmol @ photons m^{-2} s^{-1}, 5-20°C

Domestication

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Experimental Domestication Studies

Now that cultures have been obtained from the native species, we are in the process of moving to experimental commercial cultivation. Hard-shell clam shells (*Mercenaria mercenaria*) and sea-scallop shells are being inoculated with conchocelis. Investigations into the development of the conchocelis and control of conchospore formation and release are underway (Fig. 3). For some of the taxa (e.g. *P. amplissima, P. leucosticta and P. purpurea*) this is significant, since it is the first time that cultures have been successfully cycled to produce mature thalli using conchocelis-seeded shells. In laboratory culture, these nori strains are fast growing, with conchospore maturation into the leafy phase requiring less than 35 days to first harvest. The productivity and nitrogen uptake of these plants make them an excellent choice for eutrophication abatement and polyculture, while providing a high-value product upon harvest.

The project has provided fundamental baseline information regarding the taxonomic status and ecological requirements of indigenous *Porphyra* species. Further experimentation will help to isolate strains with the most advantageous characteristics for a commercially viable crop such as blade length, appropriate maturation periods and nutrient requirements for a particular site and unique pigment composition. The most promising plants are now being made available for grow-out at the Coastal Plantations International, Inc. facility in Eastport, Maine in the spring of 1999. This is an important step in the establishment of a successful seaweed aquaculture industry in Northeast America.

Notes and References

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Calendar

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May 2-6, 2000 — **Nice, France**

Aqua 2000, "Responsible Aquaculture in the New Millennium." The European Aquaculture Society will be joined with the World Aquaculture Society for this meeting, which will be held in the Acropolis Convention Centre in Nice. The conference will cover a full range of aquaculture species and topics and will include an exposition showing the latest technology and services available to the aquaculture industry. A special thematic session running the full length of the conference will focus on responsible aquaculture — Can it be accomplished? How should it be accomplished? A compendium of the presentations and an analysis of the information with conclusions will be published after the meeting. For information in the USA contact John Cooksey, Tel: 1+425-485-6682; Fax: 1+425-483-6319; e-mail: worldaqua@aol.com. In Europe contact Hilde Joncheere, Tel: +32-59-32-38-59; Fax: +32-59-32-10-05; e-mail: eas@unicall.be.

August 20-24, 2000 — **St. Louis, Missouri USA**

130th American Fisheries Annual Meeting. Adams Mark Hotel. For information, contact Betsy Fritz. Tel: +1-301-897-8616, ext. 212.

January 21-25, 2001 — **Orlando, Florida USA**

Aquaculture 2001. The next triennial meeting of the National Shellfisheries Association, the Fish Culture Section of the American Fisheries Society and the World Aquaculture Society, will take place at Disney's Coronado Springs Resort in Orlando. It is now in the planning stages. Details will be available from the World Aquaculture Society, 143 J.M. Parker Coliseum, Louisiana State University, Baton Rouge, Louisiana 70803 USA. Tel: 1+388-3137; Fax: 1+388-3493; e-mail: wasmas@aol.com, or John Cooksey, Tel: 1+425-485-6682; Fax: 1+425-483-6319; e-mail: worldaqua@aol.com.