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Porphyra Cell Suspension Cultures, Novel Biotechnological Approach for the Production of Hoshi-nori

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Abstract Species of *Porphyra* are the marine algae most commonly used for human consumption in the Orient. Recently, both natural and man-made threats to the nori crop have been encountered. Considering the potential for further loss of *Porphyra* habitat through industrial and urban development, and the risk of major localized damage from oil spills or other marine accidents, it is reasonable to consider alternative modes of hoshi-nori production which could be implemented using methods of cellular and molecular biotechnology.

A few separate clonal cell-suspension cultures (CMAC-25, CMAC-40, etc.) from regenerated protoplasts of *Porphyra linearis* have been established, and one cell-line (CMAC-25) has been maintained for more than seven years. Several aspects of physiology, growth rate and culture conditions have been studied for these cell-lines. In some cases it was possible to regenerate the leafy-thallus, pigmented callus-like tissue and the conchocelis.

It is proposed that *Porphyra* cells grown in large-scale cultures could be concentrated by filtration or centrifugation, and dried to form hoshi-nori directly. Using this biotechnology, complete control over culture conditions would enable sustainable production of a uniform, clean and high quality unialgal product, irrespective of season and climate. Although the economics of such a process are unknown, large-scale cell production would not require many of the labor-intensive steps now involved in hoshi-nori production. Cultivated cells might also be used in preparing flavouring pastes, jams and food additives.

Key words: *Porphyra*, nori, hoshi-nori, cell-suspension, mass cell culture, aquaculture, callus-like tissue, red algae, seaweed, *P. linearis*.

Porphyra, a genus of marine red algae, is utilized mainly as a human food supplement in the Orient. These algae, known in Japan as "nori", in China as "zi-cai", (meaning purple vegetable) and in Ireland and the UK as "purple laver", are the most valuable algae of commerce (Arasaki, 1982; Miura, 1975; Okazaki, 1971; Oohusa, 1993; Tseng and Fei, 1987). Worldwide annual sales of *Porphyra* products are estimated at 1–2 billion U.S. dollars (Jensen, 1993; Oohusa, 1993; Tseng and Fei, 1987). At present, the North American retail value of imported *Porphyra* is $20–30 million (U.S.) per year, with a projected growth of 10% per annum (Merrill, 1993).

A number of *Porphyra* species (Table 1) have been reported in northeastern Pacific regions (Yoshida *et al.*, 1995; Kurogi, 1972; Tseng *et al.*, 1978; Dogma *et al.*, 1986). Those currently cultivated in Japan, China, Korea and the Philippines are: *P. yezoensis*, *P. tenera*, *P. haitanensis*, *P. pseudolinearis*, *P. kuniedae*, *P. akasakae*, *P. seriata*, *P. kwangtungensis* (*P. guangdongensis*), *P. crispata*, *P. vietnamensis* and *Porphyra* sp. (Tseng, 1981; Oohusa, 1993) The first three species are the most commonly cultivated in their regions (Tseng, 1981) and account for approximately 90% of the world's commercial supply (Jensen, 1993; Oohusa, 1993; Tseng and Fei, 1987). The market value is dependent upon the species, quality and the form of the finished product,

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Table 1. List of *Porphyra* species located in Japan, China, Philippines and Canada.

PORPHYRA SPECIES	Japan[1]	China[2]	Taiwan[2]	Philippines[3]	Canada[4]
P. crispata Kjellman	X	X	X	X	
P. okamurae Ueda	X				
P. suborbiculata Kjellman	X	X	X	X	
P. dentata Kjellman	X	X	X		
P. yezoensis Ueda	X	X			
P. kinositaie (Y. et T.) Fukuhara	X				
P. moriensis Ohmi	X				
P. kurogii Lindstrom	X				
P. ishigecola Miura	X				
P. kuniedae Kurogi	X				
P. lacerata Miura	X				
P. tenuipedalis Miura	X				
P. seriata Kjellman	X				
P. katadae Miura	X				
P. tenera Kjellman	X	X			
P. akasakae Miura	X				
P. angusta Ueda	X		X		
P. pseudolinearis Ueda	X				
P. ochotensis Nagai	X				
P. irregularis Fukuhara	X				
P. crassa Ueda	X				
P. marginata Tseng et T. J. Chang		X			
P. katadae Miura var. *hemiphylla* Tseng et T. J. Chang		X			
P. haitanensis T. J. Chang et B. F. Zheng		X			
P. kwangtungensis Tseng et T. J. Chang		X			
P. vietnamensis Tanaka et P. H. Ho		X			
P. thuretii Setchell et Dawson in Dawson					X
P. nereocystis Anderson in Blankinship and Keeler					X
P. gardneri (Smith et Hollenberg) Hawkes					X
P. brumalis Mumford					X
P. perforata J. Ag.					X
P. torta Krishnamurthy					X
P. abbottae Krishnamurthy					X
P. maculosa Conway					X
P. fucicola Krishnamurthy					X
P. pseudolanceolata Krishnamurthy					X
P. sanjuanensis Krishnamurthy					X
P. umbilicalis (Linn.) J. Agardh					X
P. leucosticta Thuret in Le Jolis					X
P. linearis Greville					X
P. onoi Ueda	X				
P. pseudocrassa Yamada et Mikami	X				
P. punctata Yamada et Mikami	X				
P. simithii Hollenburg et Abbott					X
P. kanakaensis Mumford					X
P. amplissima (Kjellman) Setchell et Hus	X				X
P. variegata (Kjellman) Kjellman	X				
P. tenuitasa Fukuhara	X				
P. occidentalis Setchell et Hus	X				
P. papenfussii Krishnamurthy					X
P. schizophylla Hollenberg in Smith and Hollenberg					X
P. variegata (Kjellman) Hus					X
P. miniata (C. Ag.) C. Agardh					X
P. purpurea (Roth) C. Agardh					X

[1]Yoshida *et al.*, 1995, [2]Tseng *et al.*, 1978, [3]Dogma *et al.*, 1986, [4]Garbary *et al.*, 1980, Bird and McLachlan, 1992.

with Japanese hoshi-nori sheets receiving the highest price.

Hoshi-nori is the name used to describe the dried rectangular, paper-like sheets of foliose *Porphyra* thalli cultivated and harvested from the sea (Okazaki, 1971; Miura, 1975; Oohusa, 1993). Nori crops are harvested, washed, chopped into pieces and processed into this product through a series of steps using complex modern machinery. Hoshi-nori is mainly used to wrap rice in preparing sushi in the traditional Japanese daily diet (Okazaki, 1971; Miura, 1975).

The use of *Porphyra* as a food and medicine on the southeastern coast of China was first recorded during 960–1279 A. D. At that time, the coastal people of Fujian Province selected "zi-cai" as one of the best goods presented to the emperor annually (Tseng, 1981). Its use in Japan was reported over a thousand years ago (Arasaki, 1982; Mumford and Miura, 1988). In an effort to increase crop production, it is reported that early in the 1600s the coastal people of Japan collected *Porphyra* spores in nature by inserting bundles of bamboo twigs called "hibi" (Okazaki, 1971; Miura, 1975; Arasaki, 1982), while the Chinese in southeastern coastal waters used a "rock cleaning" method, applying unslaked lime to stones to remove unwanted species just before the season of mass spore release, thus permitting conchospores to recolonize and germinate (Tseng, 1981). The Japanese "hibi" method provided more area for released conchospore attachment than the simple Chinese rock cleaning method for collecting spores. Because such primitive methods depended entirely on the natural spore release and settlement for success, *Porphyra* production fluctuated greatly from year to year.

Early in the present century, the horizontal floating net culture developed by Japanese scientists and aquaculturists, and promoted by their government, was introduced to replace the old "hibi" method (Okazaki, 1971; Miura, 1975; Oohusa, 1993). In China, shortly after the new government was established in 1949, scientists began to develop *Porphyra* aquaculture in coastal waters to help fishermen propagate crops from the sea (Tseng, 1981).

Significant improvements became possible in the 1950's after Kathleen Drew's discovery that conchocelis was the alternate generation of the foliose thallus phase of *Porphyra* (Drew, 1949). The subsequent development of nori as a major cultivated crop in Japan proceeded rapidly when the growers were able to control the production of "seeds" and develop techniques to store "seedlings" thereby making juvenile plantlets available over an extended season (Kurogi, 1953a; 1953b; 1961; Okazaki, 1971; Miura, 1975; Oohusa, 1993). Commercial cultivation of nori became truly modernized during the 1960s when floating-net cultivation techniques were further improved (Okazaki, 1971). The floating-net techniques started in the near-shore intertidal zone using poles or pillar methods, as well as the intertidal raft method. Space limitations in shallow water areas forced aquaculturists to increase the use of the deep water floating raft method to increase culture area in the sea. A closely integrated system of aquacultural technology and modern processing machinery for hoshi-nori forms the basis for the present industry in Japan and is being followed elsewhere (Tseng, 1981; Kain, 1991; Oohusa, 1993).

Meanwhile, the rapid development of heavy industries in Japan during the 1960s led to severe pollution of the air and sea and consequent loss of *Porphyra* habitat. With high intensity cultivation comes another serious problem, diseases of the nori crop. For example, a chytrid-like parasite attacked the nori crops in Tokyo Bay in epidemic proportions in 1959–1960 (MacFarlane, 1968; Ogata, 1975). This region, formerly a major site of *Porphyra* cultivation, became so greatly polluted that it was necessary to abandon the "hibi" cultivation grounds that had been used successfully for centuries (Aruga, 1990). Dredging and filling of near-shore areas, increased ship traffic and oil pollution have further reduced habitat for nori propagation. There have been reports that in the 1991–1992 season (Chen *et al.*, 1990a), the Japanese production of seaweeds was reduced by almost 50% owing to a combination of poor weather conditions and pollution. Similar circumstances are also developing in other Asian regions.

In North America, there is growing interest

in cultivation of *Porphyra* owing to the rapid increase in demand for nori products now imported from Asia. This high rate of nori importation is partly driven by an increased awareness of the dietary benefits of consuming marine algae. Health conscious consumers are turning from animal products and are searching for diverse vegetable alternatives.

Small-scale commercial trials of transplanted *P. yezoensis* utilizing Japanese aquaculture technology were successfully conducted in Puget Sound, Washington State (Mumford and Hanson, 1987; Waaland *et al.*, 1986). Further expansion of the culture of nori in Puget Sound was not possible for socio-political reasons. Similar attempts in northeastern Maine by Coastal Plantations to propagate *P. yezoensis* have been made since 1993. Commercial success of this venture remains to be established (Chen *et al.*, 1995).

The successful transfer of aquaculture technology for the culture of *Porphyra*. from Japan to Atlantic Canada is problematic for a number of reasons. First, coincidence of photoperiod and temperature for optimal nori culture differs significantly between locations in Japan and Atlantic Canada. The Japanese species are usually grown in comparatively warmer water than is found along the coasts during the winter season. Second, transplanted "seeded nets" from the Orient have the potential for contaminating the local environment by introducing undesirable organisms such as fungi, bacteria and other disease-causing agents as well as foreign marine plant and animal species. Such chance introductions may be deleterious to the local marine community. By comparison, utilizable native species that are already adapted to the local environment will be more suitable for propagation under local conditions.

At the Institute for Marine Biosciences (IMB) of the National Research Council of Canada, scientists developed extensive knowledge on the ecology, taxonomy, life history, natural resource production, cultivation, as well as the chemical composition of marine plants. Especially, IMB researchers haveaccumulated much basic scientific knowledge of *Porphyra* including life history, ecology, distribution and aspects of chemistry and physiology. This is valuable information on which to develop *Porphyra* spp. for possible commercial nori production. While none of the currently commercial species is endemic to the Atlantic coasts, six species of *Porphyra* are reported in the Maritime Provinces (Bird and McLachlan, 1992). One of these, *P. linearis*, was identified early as a potential source of high quality nori with superior flavour and texture comparable *P. tenera* and *P. yezoensis* (Craigie, 1971; McLachlan *et al.*, 1972; Guptill, 1994). The factors responsible for the desirable flavour of *P. linearis* include a relatively high concentration of isofloridoside and of several free amino acids such as alanine, glutamic acid and glycine, as well as the absence of amides that impart unpleasant, fishy odours (McLachlan *et al.*, 1972). The characteristics of high quality *Porphyra* (taste, colour and texture) possessed by *P. linearis* would be advantageous in providing a competitive edge in both the local and export markets where it would compete directly with an established product (Guptill, 1994).

Porphyra linearis is a winter annual, growing from late October to early April along the north Atlantic Canadian coasts at exposed sites in the upper intertidal spray zones (Edelstein and McLachlan, 1966). As the fronds mature they produce carpospores and, through an alternation of generations, the species spends the rest of the year as a microscopic "conchocelis" phase (Bird *et al.*, 1972; Bird, 1973). Conchospore release appears to occur in late summer as the seawater temperature reaches approximately 13–14℃ and can continue to some degree until the following June (Bird, 1973). The life cycle of this species has been demonstrated for five generations under controlled environmental conditions (Bird *et al.*, 1972). In most cases, 1–3 months elapsed between transfer to 13℃ and the release of conchospores. Considerable variations in the growth rate, colour, degree of branching, morphology of conchosporangia and the ability to release conchospores suggest that genetically different strains may exist within this species.

Available information indicates that the fronds of *P. linearis* do not produce neutral spores as do the commercially cultured Ori-

ental *Porphyra* species (Mumford and Miura, 1988). Because of the lack of neutral spores in *P. linearis*, repeated harvests of vegetatively produced fronds during the growing season can not be expected. In an effort to create "artificial neutral spores", studies were carried out on the formation of isolated protoplasts from fronds of *P. linearis* (Chen *et al.*, 1988; Chen *et al.*, 1994). The mechanisms by which the protoplasts regenerate into either new fronds or, in some cases, conchocelis filaments are not clear. Further studies are required to elucidate the development of fronds from isolated protoplasts.

Prior to our work, attempts had not been made to domesticate this potentially superior species for commercial nori production. Recently, I conducted studies on protoplast isolation and morphogenesis of *P. linearis* and established a few *Porphyra* cell lines (Figs. 1, 2 & 4). In traditional hoshi-nori production, the fronds are harvested from the sea and manufactured into dry sheets. It should be possible to use concentrated cell suspensions instead of *Porphyra* thalli to make hoshi-nori directly (Chen *et al.*, 1990a). The achievement of cell-suspension culture of *P. linearis* (Chen, 1989) and the establishment of clonal cell lines from regenerated protoplasts of *P. linearis* might provide the opportunity to develop an alternative method of propagation similar to mass culture of unicellular algae or vascular plant cell suspension cultures.

Before proposing this novel biotechnology using mass cell culture as raw material for hoshi-nori production (Chen *et al.*, 1990b), I conducted extensive studies on isolated cell lines to investigate their growth rate, morphogenesis, cell-division, and cell stability in culture. These cell lines have been maintained in culture for several years under various cultural conditions on D-11 medium (Chen, 1988) without forming either leafy-thallus or conchocelis. The isolated cells derived from protoplasts of *P. linearis* showed a range of sizes from 5 to 150 μm in diameter (Fig. 1). However, most were small cells (10–35 μm) with relatively dense plastids (Chen *et al.*, 1990a, 1990b). These isolated cells (Fig. 1) resembled vegetative cells of the leafy-thallus. When they were transferred to suitable culture conditions, they divided and formed a group of cells. Carefully examined under high magnification, the cells appeared as individuals loosely connected to form groups without any outer wall. If the culture container was shaken gently, they spread into a suspension of individual cells.

At the beginning of cell-division, the cells elongated and became peanut-shaped (Fig. 3) with a furrow appearing around the equatorial region. As cell-division continued, the furrow gradually deepened until the cell appeared to have been pinched in two. As the intercellular connection between the two developing daughter cells became longer and narrower.

In single cell microculture, cell-division took place every 24–30 hours under optimum condition. However, with a density of 10^4 cells ml^{-1} of culture at 15℃, 10 μmol of quanta $m^{-2}s^{-1}$ and 10 : 14 (L : D) photoperiod, I could only achieve a doubling time of approximately 52 h. The doubling time will lengthen as the cell density increases under the same culture conditions. This longer doubling time relates to increased cell density in culture, perhaps related to a nutrient decrease in the medium, or to reduced light penetration, or perhaps to deleterious waste products in the culture medium. Obviously, improvement of the cell propagating techniques is required.

Cell suspension cultures have been propagated continuously for several years as populations of individual cells without their changing into leafy-thallus, conchocelis, or clump-forms. However, it is possible to manipulate the culture conditions and to induce a single cell to form either a leafy-thallus (Fig. 7), a clump (Figs. 5, 6 & 7) or a conchocelis filament. When some of these small cells were transferred to TC-11 medium (Chen, 1988) and were cultured at a low temperature (< 10℃) with a short photoperiod (< 12 h) at approximately 20 μmol of quanta $m^{-2}s^{-1}$ for about 3 months, they formed flat blades that appeared to resemble leafy-thalli of conchosporelings. However, if a culture from a mixture of large and small cells grown under conditions with > 10℃, > 16 h with a photon flux density of 50 μmol $m^{-2}s^{-1}$ for 2 months, the cells developed almost exclusively into conchocelis filaments. I do not yet

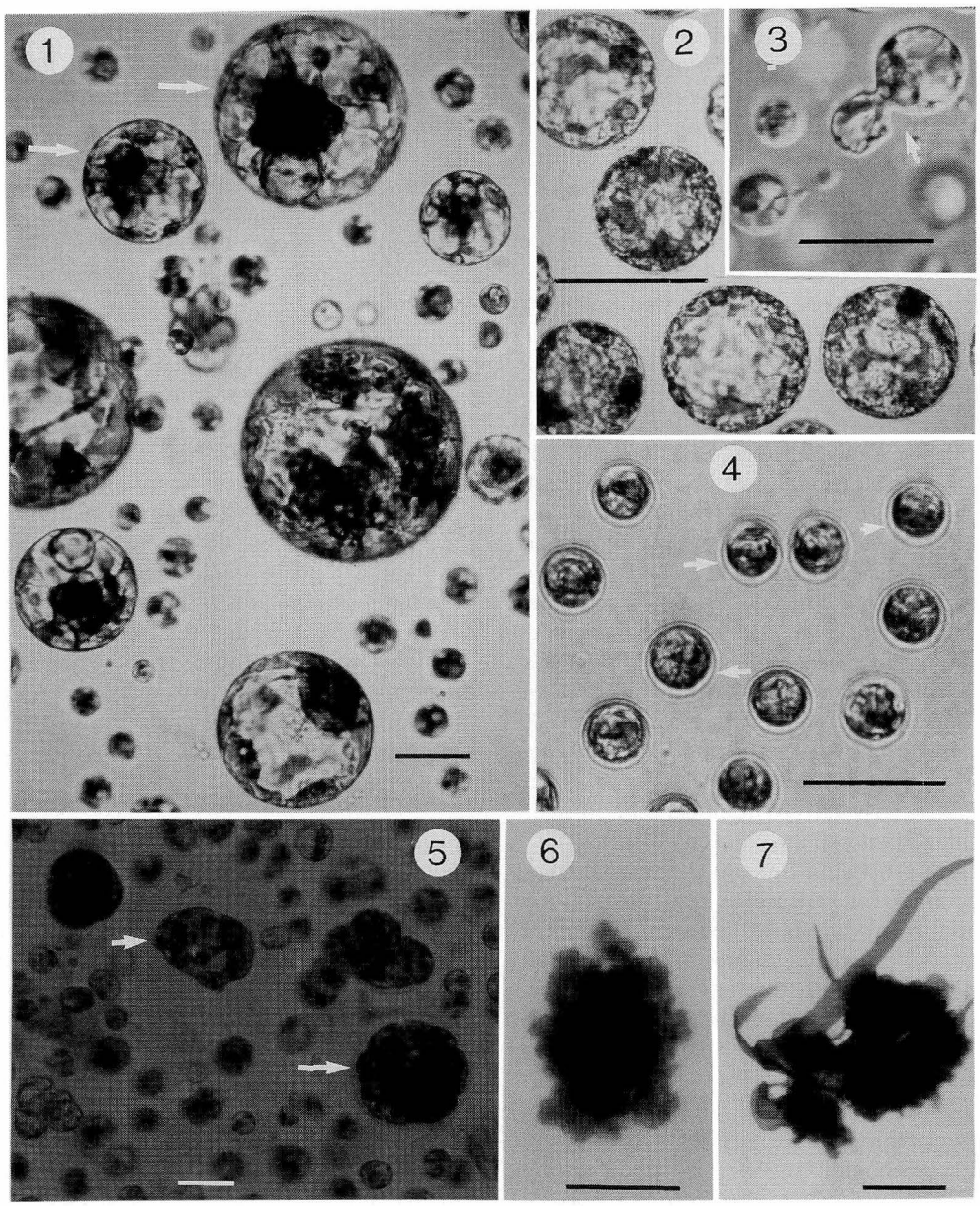

Fig. 1. 1. Various sized cells derived from isolated proptoplasts of *Porphyra linearis* leafy-thallus. (Arrows showing the normal stellate plastid characteristic of *Porphyra*). Scale bar is 50 μm for 1–5. 2. CMCA-40 clonal cell line with uniform cell size. Note cells showing discoid plastids. 3. Cell division: Arrow shows the furrow shape (Nomarski microscope). 4. CMCA-25 clonal cell line showing cell wall (Arrows). 5. Cells initiating callus-like tissues without thickened cell wall (Arrows). 6. Clump (callus-like tissue) with pigmentation. Scale bar is 500 μm for 6 & 7. 7. Leafy-thallus developed.

understand the mechanism for the formation of either the leafy-thallus or the conchocelis (Chen 1989) and further studies are needed.

In addition, several clonal cell lines have been evaluated under a variety of culture conditions for suitability for cell-suspension culture (Chen *et al.*, 1990a). Isolated protoplasts of *P. linearis* thallus that did not regenerate new plant-forms were propagated under conditions permitting continuous division to produce a group of cells showing a range of size from 5 to 150 μm in diameter. Among these cells, there was a group of fairly uniform cells about 25–30 μm in diameter resembling vegetative cells of the leafy-thallus. A stable clonal cell line (CMAC-25, Chen *et al.*, 1990a) was established after 10 months of repeated propagation from a single cell isolation using a micro-culture system. Other cell lines evaluated were unstable, dividing unequally or producing unpigmented cells or aberrant forms, some in callus-like pigmented clumps. To obtain these clumps, the CMAC-40 cell line was initially suspension-cultured for 24 weeks in D-11 medium under a constant temperature of 15℃ with an 8 h photoperiod, at a photon flux density of 20 μmol $m^{-2}s^{-1}$. Fresh D-11 medium was replaced every other week. After 24 weeks, this cell line suspension-culture was transferred for an additional eight weeks to D-11 medium with the addition of 10^{-7} mM kinetin, 10^{-6} mM NAA and 10^{-8} mM 2,4-D (changed weekly) under a constant temperature of 7℃ with a photoperiod of 8 : 16 (L : D), at a photon flux density of 16 μmol $m^{-2}s^{-1}$. Some of the cells were found to be dividing into two cells within a single cell wall, they gradually became multicellular irregular callus-like tissue (clumps) with normal pigmentation. When propagated in the above culture conditions for prolonged periods, these clumps generally increased in size, although in some cases it was noticed that portions of clumps were either split or had broken off resulting in an increased number of clumps (Chen, unpublished data).

There is a longstanding importance of hoshi-nori in the Japanese diet, and of "zi-cai" in Chinese food. Recognized natural and man-made threats to the crops and the potential for further gradual or catastrophic loss of Asian *Porphyra* habitat put the crops in potential jeopardy. At the same time there are both increased demands and fewer cultivated areas. This made it reasonable to consider novel biotechnological alternatives to the current mode of hoshi-nori production. With the establishment of clonal cell lines and laboratory-scale axenic cell-suspension cultures of *P. linearis* derived from isolated protoplasts (Chen, 1989; Chen and McCracken, 1993), it becomes possible to suggest that an alternative method of propagation, similar to mass culture of unicellular algae or vascular plant cell-suspension cultures might be used to supplement or even replace conventional *Porphyra* aquaculture. Such novel biotechnological methods would permit the production of hoshi-nori sheets directly through the propagation of mass cell-suspension cultures under suitable culture conditions. We have already successfully induced cells from small scale cell-suspension culture to develop into multi-cellular, irregularly shaped foliose thalli or callus-like pigmented "clumps" (Chen, unpublished data) or plantlets (Chen, 1989). These can also be used to produce hoshi-nori products directly.

Use of such an approach would eliminate many of the labour intensive steps in the propagation of raw nori crops from conventional *Porphyra* mariculture. It is now conceivable that *Porphyra* cell-suspension cultures, derived from isolated protoplasts, can be grown in large-scale controlled indoor tank cultures propagated to a maximum cell density, or to be converted to form clumps or young plantlets. These could be harvested by centrifugation or filtration, dried and used to form hoshi-nori directly. This revolutionary approach for hoshi-nori production offers several potential advantages over conventional aquacultural methods in the sea:

1) Complete control over culture conditions would enable sustainable production of a uniform product, irrespective of season, climate and other natural conditions.

2) The necessarily near-axenic culture conditions required would by definition, eliminate problems caused by epiphytes, fungi, bacteria or other pathogens.

3) The potential for pollution and toxins

to threaten the crops could be eliminated resulting in improved consumer confidence in the product and a higher retail price.

4) Only a small space would be needed for indoor tank cultures.

Industrial propagation of cell suspensions would obviate the need for many labour-intensive steps such as:

a) Collecting carpospores from fertile leafy-thalli during January to March.

b) Seeding carpospores on oyster shells and manipulating the spore germination to give rise to conchocelis.

c) Propagating and maintaining the conchocelis in indoor tank cultures.

d) Manipulating conditions for conchosporangia formation and releasing conchospores from their filaments.

e) Mass seeding of conchospores on the nets.

f) Initiating the plantlets of seeded nets in controlled indoor culture tanks.

g) Freezing and storing the frozen nets bearing germinated sporelings.

h) Awaiting suitable weather to place nets with germinated sporelings in the sea.

i) Controlling epiphytes, grazers and diseases on the growing plants.

j) Fertilizing the plants in the sea during the growth period.

k) Collecting and harvesting the crop from the sea.

l) Washing and cutting the intact thalli into pieces for hoshi-nori production.

I have recently conducted a series of systematic experiments (Chen, unpublished data) to determine growth rates, cell-division and attainable cell density; effects of nutrient composition, pH, mechanical stirring or air agitation of culture media and the stability of cell-lines of *P. linearis*. In summary: 1) The growth rate increased with temperature up to 20℃ at a given photoperiod and photon flux density, however, the differences in growth rates with temperature were less pronounced than those found between different photoperiods. 2) When the cell line was cultured axenically in D-11 medium at 20℃ with a 16 h photoperiod under 20 μmol of quanta $m^{-2}s^{-1}$ at a cell density of 1.5×10^4 cells ml^{-1}, the doubling time (4 days) showed significant improvement compared with the early results (not axenic). This suggests that the doubling time can be reduced by choice of culture conditions. 3) Cell density increased from an initial number of 9.5×10^4 cells ml^{-1} to 7.2×10^5 cells ml^{-1} within 9 days in a 2 l volume of medium agitated with air and with pH controlled (7.5–8.0) while being cultured at 15℃, 16 : 8 (L: D) 20 μmol of quanta $m^{-2}s^{-1}$. 4) Although full strength D-11 medium could maintain growth longer, cell number (initial cell density of 1×10^3 cells ml^{-1}) was increased significantly (183% better) using the medium diluted by 50% with seawater at an early growth stages (3 days). 5) At a higher cell density (7.2×10^5 cells ml^{-1}), cell number increased initially but thereafter decreased rapidly (Chen, unpublished data). This confirmed that a constant supply of nutrients could sustain a higher cell culture density. Although this information is preliminary, these conclusions move us one step closer to the possibility of using mass *Porphyra* cell suspension cultures to produce hoshi-nori directly. More research is still needed before setting up large scale mass cell-suspension cultures for commercial production.

In addition to the use of cultured *Porphyra* cells for production of hoshi-nori products, and other foods such as jam, paste, candy etc., appropriate cell lines might also serve as "seed" stock for more conventional nori aquaculture.

It is possible also that clonal cell lines such as these can serve as reliable sources of the large number of cells likely to be required for systematic investigation of intra- and interspecific cell fusion and molecular transformation to improve the quality of nori cell lines or *Porphyra* thallus and other nori products. By interspecific cell fusion, organelles and genomes can be introduced into novel cytological backgrounds for investigation of parasexual reactions and selection of desired phenotypes, These can be re-isolated as protoplasts to re-establish improved cell lines. If nuclei are fused successfully, similar possibilities are opened for traits specified by nuclear genes.

Techniques for *Porphyra* protoplast electrofusion have also been initiated and some of the procedures being developed show pro-

mise (Chen *et al.*, 1995). These techniques may also be used to improve present cell-lines for fast growth and/or better quality. For DNA transformation, promoter elements and other regulatory features of red algal nuclear genes will be required for successful transformation.

In conclusion, the increase in demand for a quality product by consumers aware of the dietary benefits of marine plants, the desire to reduce labour input in nori production and the longstanding importance of hoshi-nori in the traditional diet of Japanese and other Asian peoples require that nori production be expanded. In addition, the deterioration of environmental conditions along much of the Asian coastline precludes significant further expansion of conventional nori mariculture. The technology of mass cell suspension culture to produce hoshi-nori directly is a potential alternative to the traditional conventional nori propagation. Although the economic benefits from the mass cell suspension culture cannot be assessed at this time, the technology can offer a steady supply of clean, uniform, high quality raw material for nori production, irrespective of season and climate. The product may also command a premium retail price if consumers can be guaranteed a product free from pollutants.

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アマノリの細胞懸濁培養：バイオテクノロジーによる乾海苔生産の新展開

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紅藻アマノリ属植物は東アジアにおいて最も普通に食されている海藻である．近年，海苔の生産は自然災害と人為的災害の脅威にさらされている．工業や都市の発達によって，特に，局地的に発生する油の流出や海洋事故などによる被害とその危険性によって，アマノリ属植物の生育地と養殖海域はさらに減少していることを考慮すれば乾海苔生産の新しい方法について検討することは理にかなっている．

Porphyra linearis のプロトプラストから再生し分離された数系統のクローナル懸濁培養細胞系 (CMAC-25, CMAC-40 等) がつくられてきた．そのうちの一つの細胞系 (CMAC-25) は 7 年以上も保存培養され，その成長率や培養条件といった生理学的な側

面が研究されてきている．その培養細胞は，葉状体，色素を有したカルス状の組織，コンコセリスに再生することが可能であった．

大量培養によって生産されたアマノリ属植物の細胞を濾過や遠心分離によって濃縮し，直接乾燥させて乾海苔生産をおこなう方法を提案する．このバイオテクノロジーを使い，培養条件を完全にコントロールする乾海苔生産の方法は，季節や気候の変動に左右されず均一，清浄で，かつ高品質な混入物のない乾海苔を持続的に生産することを可能にする．この方法による海苔生産の経済性については充分に明らかにされてはいないが，この大量細胞生産による方法は，現在の乾海苔生産が抱える労働集約的な手段の多くを必要としないであろう．そして，培養された細胞は，香料，ジャム，食品添加物など多方面に利用される可能性がある．

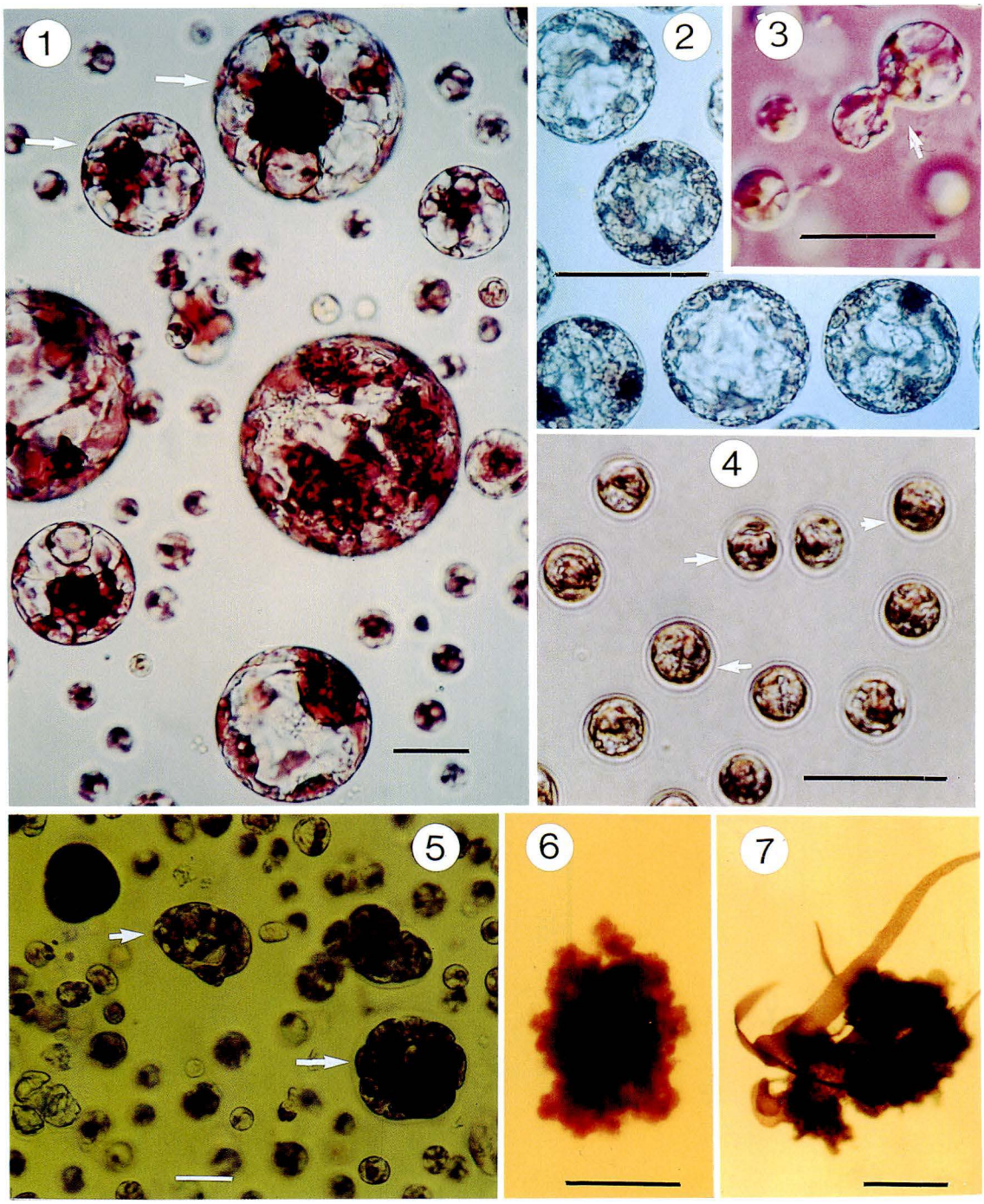

Fig. 1. Various sized cells derived from isolated protoplasts of *Porphyra linearis* leafy-thallus. (Arrows showing the normal stellate plastid characteristic of *Porphyra*). Scale bar is 50 μm for Figs. 1–5.
Fig. 2. CMCA-40 clonal cell line with uniform cell size. Note cells showing discoid plastids.
Fig. 3. Cell division: Arrow shows the furrow shape (Nomarski microscope).
Fig. 4. CMCA-25 clonal cell line showing cell wall (Arrows).
Fig. 5. Cells initiating callus-like tissues without thickened cell wall (Arrows).
Fig. 6. Clump (callus-like tissue) with pigmentation. Scale bar is 500 μm for Figs. 6 and 7.
Fig. 7. Leafy-thallus developed.