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Model Plants in Marine Biotechnology

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Abstract The genus *Porphyra*, consisting of 133 species includes several economically valuable species (i.e. *P. yezoensis*, *P. tenera*, *P. pseudolinearis* etc.). They are predominantly consumed and cultivated in East Asian countries such as Japan, Korea and China, and they are regarded as a big commercial market today. In addition to the industrial importance, *P. yezoensis* is currently regarded as a feasible candidate for a model plant in marine biotechnology, therefore there are a wide range of studies being undertaken: strain-preservation, development of mutant strains and genetic analysis and exhaustive molecular analysis including EST and macro/micro array. Focusing on the activities of our research group, current situation and future perspectives in marine biotechnological studies using *P. yezoensis* will be discussed in this mini review.

Key words : marine biotechnology, model organism, *Porphyra yezoensis*, red alga

Introduction

Marine algae are a diverse group of over eleven thousands species ranging in size from the microscopic unicellular plankton to macroscopic multicellular benthos. Marine macroalgae collectively known as seaweeds are those algae large enough to be seen with the naked eye, and they consist of the three major phyla, i.e. Chlorophyta (green algae), Chlomophyta (brown algae) and Rhodophyta (red algae). The members of marine macroalgae except Chlorophyta (green algae) have never got to land, most of them, if not all, have adapted themselves to marine circumstances and accomplished their unique evolution. Since survival strategy of marine macroalgae, i.e., photosynthetic mechanism, cell component structure, life cycle pattern, halophilism, etc. differs from those of land plants, they might have various characteristics and biological functions peculiar to marine circumstances. It is desirable to establish some proper model organisms for efficient promotion of advanced studies of their own characters and special functions. In a recent review [12], various kind of taxa were proposed as candidate macroalgal models for genomics, e.g. the genus *Ulva* as a Chlorophytic candidate, the genus *Ectocarpus* as a Chlomophytic candidate and the genus *Porphyla* as a Rhodophytic candidate. Post-genome analysis became to the main stream in modern biology using eukaryotic organisms including yeast, nematode, fly, mouse, human, etc. In plants, genome analyses had been done for several land plants, such as *Arabidopsis, Oryza* or *Physcomitrella* and unicellular microalgae, such as *Chlamydomonas*, *Cyanidioschyzon* or *Thalassiosira*. In addition, large scale-genome analysis of multicellular macroalgae such as *Ectocarpus* [8] or *Porphyra* [9] is recently promoted in a world-wide level. It has already been organized some genome consortium for *Ectocarpus* in EC countries and *Porphyra* in Asian countries to which highly amounts of national and/or international budget is invested.

The present status of infrastructure arrangements for advanced studies on *Porphyra* as a model organism in marine biotechnology will be introduced in this mini-review, featuring the activities of our research group.

Porphyra as a model organism

The members of *Porphyra*, a genus of marine macroalgae, are distributed all over the world, and of which there are 133 species recognized in the world and 29

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species recognized in Japan [15]. *Porphyla* includes several economically valuable species, *P. yezoensis*, *P. tenera*, etc., they are the most valuable marine crops in the world. Their recent annual yields are about 10 billion sheets, and recent annual sales of their products are estimated at multibillion U. S. dollar.

Porphyra has recently received great interest as a model plant for fundamental and applied studies in marine sciences. *Porphyra* has a unique dimorphic life cycle which consists of a leafy gametophytic generation and a filamentous sporophytic phase(Fig. 1). Some species of *Porphyra* have an asexual cycle consisting of repetition of leafy gametophytes through monospores. It completes its life cycle in laboratory culture within a few months and has a small number of chromosomes (2-7) in the genus *Porphyra* ($1.3\text{-}5.3\times10^8$bp) are in the same order of magnitude as those of *Arabidopsis thaliana*. Genetic analyses, including both classical and modern molecular studies are in progress.

The members of our research group are performing the following subjects in order to make *Porphyra* a sophisticated model organism: strain-preservation and cryo-preservation [5], establishment of mutant cell lines [11], development of transgenic study [3], genome project including genetic map [7], EST [1,6], DNA macro/micro array [4], etc. Among the subjects aforementioned, a genetic transformation system, a DNA marker for the genetic map and ESTs, which are the innovative trial in marine macroalgae, will be introduced.

The marine red alga *P. yezoensis* has been proposed as a model plant for physiological and genetic studies in seaweeds because of its biological and economical importance. However, the progress of molecular biological studies using gene transfection and genetic transformation systems has been hindered by difficulties in the expression of foreign genes in *P. yezoensis* cells. To overcome this situation, we developed a transient gene expression system to monitor gene expression in *P. yezoensis cells. An artificial β -glucuronidase (GUS) coding region was synthesized to adapt it to the codon usage of P. yezoensis* (PyGUS) and then evaluated for efficiency as a reporter of transient gene expression by particle bombardment. We also demonstrated the importance of using the promoter of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene from *P. yezoensis* for efficient expression of PyGUS, because the cauliflower mosaic virus (CaMV) 35S promoter, which has been successfully used for monitoring gene expression in nuclei and chloroplasts of higher plants, was less active in *P. yezoensis* cells [3]. Therefore, the lack of knowledge about differences in the regulatory machinery of gene expression between *P. yezoensis* and terrestrial plants seems to be why experimental systems for monitoring gene expression were previously not developed in *P. yezoensis*. Establishment of the transient gene expression system in *P. yezoensis* could facilitate biotechnological developments in this organism.

For generalizing *P. yezoensis* as a more sophisticated model, we are also trying to develop the genetic linkage map for this alga. Recently, genetic maps based on a wide range of new molecular markers have been constructed. As an initial step for this purpose, we established several pure lines for genetic analysis and examined genetic distances among those strains. These efforts resulted in identification of two appropriate strains (named TU-2 and KGJ strains) which can be readily used in molecular genetic studies. DNA-based genetic markers are efficient and robust tools for providing the high-resolution diagnostic traits required for detailed linkage analysis [7]. In general, co-dominant molecular markers can allow more precise analysis and provide a good wealth of information. Therefore, using cleaved amplified polymorphic sequence (CAPS) analysis, we developed several kinds of nuclear gene markers.

In an attempt to search for genes related to the morphological and physiological differences between gametophyte and sporophyte of *P. yezoensis*, a large-scaled expression sequence tag (EST) analysis was carried out. Total of 10,625 and 10,154 of 5'-end sequences were generated from sporophytic [1] and gametophytic [6] cDNA libraries, respectively, and they were clustered into 4,496 nonredundant groups. Among those, only 1,013 (22.5%) sequences were classified as ESTs that commonly expressed in both generations, whereas the large proportion of EST groups were identified as being unique to either the gametophyte or sporophyte. A statistical analysis of expression profiles of the ESTs revealed that 89 and 112 highly expressed gene candidates in the gametophyte and sporophyte, respectively. Codon usage analysis in the coding regions of 101 non-redundant EST groups showing significant similarity to known genes indicated the higher GC contents at the third position of codons (approximately 80%) than the first and the second position, suggesting that

the genome has been exposed to high GC pressure during evolution. The sequence data of individual ESTs are available at the web site http:www.kazusa.or.jp/en/plant/porphyra/EST/.

Development and growth

We have been studying the development and regulation of a life cycle in *Porphyra*. During these studies, we are particularly interested in molecular biology concerning the sexual cell differentiation and determination of generations.

Life cycle and morphogenetic modes: As mentioned above, there is the heteromorphic altermation of generations between a leafy gametophyte and a filamentous sporophyte in *Porphyra*. Using a scanning electron microscope (SEM), a transmission electron microscope (TEM) and a biochemical approach, a cytological survey of the vegetative cells of the gametophytic and sporophytic phases in *Porphyra* showed that two stages differ in a number of structural characteristics: form and growth (diffuse vs apical), chloroplast number (one vs many), pit plug (absent vs present), vacuole (peripheral vs central) and cell wall structure (xylan vs cellulose). These two generations sometimes seem to be different species even though they have the same genome.

The morphogenetic modes of them are different, and this may be explained by different gene expression. Our interest focused on the developmental stage- or generation phase-specific gene expression in *Porphyra*.

Morphogenesis and symbiosis: We are further interested in the relationship between *Porphyra* and symbiotic bacteria. In axenic cultures, *Porphyra* lost its typical morphogenesis during the gametophytic phase, although it kept morphogenesis during the sporophytic phase. Some symbiotic bacteria recovered the deficient morphogenesis. Yamazaki et al. [14] found that morphogenesis-inducing bacteria isolated from *Porphyra yezoensis* restore its typical folious morphology. They isolated 324 bacterial strains from the conditioned medium of unialgal culture of the alga, and 4 strains among these, BPY-W4, -W6, -W8, and –W9 induced algal morphogenesis. Especially the thalli, when restored by BPY-W8 grew about 2,000 times larger than control after 3-months. Scanning electron microscopic observations revealed that BPY-W8 only attached to the rhizoid surfaces of the algae which were 4 to 14 days old. When the thalli that were more than 15 days old were cultured with BPY-W8, the bacteria did not attach to the algal surfaces. Their results suggest that the strain BPY-W8 may attach onto the specific algal area and development period, and that direct attachment between *P. yezoensis* and BPY-W8 is necessary for the typical growth and morphogenesis of the gametophytic thalli.

Genetic analyses in *Porphyra yezoensis*

Some of our research group is working on genetics of *P. yezoensis*. We introduce the two topics in this section; mutant strains and molecular genetic analysis.

Development of mutant strains: In *P. yezoensis*, pigment mutations have been generally used as genetic markers. Yan and Aruga [13] induced several pigmentation mutants using N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Tomimatsu et al. [11] developed a revised method of Yan and Aruga [13], and succeeded in inducing a different kind of greenish mutant strain named MBG from TU-1 strain.

Some of the mutants described above have been utilized in genetic analyses [10], however, detailed investigations at molecular level has not been reported to date. Therefore, disjunction between classical Mendelian genetics and molecular approach is a serious problem to be overcome. In addition, in the case of *Porphyra* species, most of the mutants reported to date are the pigmentation mutants. Thus, development of other kinds of mutants with some physiological defects, such as auxotrophic or sexually sterile strains would provide new insights in genetic analysis of this alga.

Molecular genetics: Cross breeding of *P. yezoensis* has long been conducted for improvement of strains since 1980s, and in the early days, pigmentation mutants were extensively used in crossing. Although pigment mutation provides an unequivocal character which enables easy confirmation of heterozygosity by color, it requires a long waiting time because visible chimeric thalli usually appear several months after fertilization. To overcome this problem, our research group has developed a new rapid method for identification of cross fertilization by two CAPS markers for nuclear genes [2]. Using these markers, we could examine heterozygosity of the conchocelis so easily and we can avoid the time consuming process. Since *P. yezoensis* is a monoecious alga in which the female and male gametes are formed in one blade, isolation of fertilized zygotes requires highly skilled techniques. Therefore, con-

tamination of self-fertilized conchocelis sometimes occurs during cross experiment, and the nuclear markers cannot distinguish those. This may lead to miscounting and fatal errors in statistical analysis in genetic studies. To solve this problem, we recently developed two CAPS markers for chloroplast and mitochondrial genomes. Using these markers, we can thoroughly exclude the self-fertilized conchocelis.

In summary, molecular genetic analysis in *P. yezoensis* is still in the dawning, however, we have prepared the two absolutely necessary tools; strains and markers. Thus, we are optimistic of stepping into the next phase of molecular genetics, now and near future.

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