Genetic information of three pure lines of Porphyra yezoensis (Bangiales, Rhodophyta) obtained by AFLP analysis

OSAMU IITSUKA,1 KAYO NAKAMURA,2 AKIYUKI OZAKI,2 NOBUAKI OKAMOTO2 AND NAOTSUNE SAGA1,*

1Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611 and 2Department of Aquatic Biosciences, Tokyo University of Fisheries, Minato, Tokyo 108-8477, Japan

ABSTRACT: Porphyra (Bangiales, Rhodophyta), which includes several valuable marine crops, has recently received great interest as a model plant for fundamental and applied studies in marine sciences. Amplified fragment length polymorphisms (AFLPs) are a robust and efficient means for genetic mapping, linkage analysis of genetic characters for breeding and population studies in land plant genomes. To examine whether AFLPs are applicable as genetic markers in the present study, we detected AFLP markers with three pure lines in order to promote genetic analysis in Porphyra yezoensis. The following five sets of AFLP primer pairs (E-AA, M-CAA) (E-AA, M-CAC) (E-AA, M-CAG) (E-AA, M-CAT) (E-AA, M-CTA) were tested with template DNAs from three pure lines and they showed a total of 227 bands. This suggests that AFLP markers are promising tools for genetic analysis in Porphyra.

KEY WORDS: AFLP, genetic marker, laver, model organism, pure line, red alga, Porphyra yezoensis.

INTRODUCTION

The members of the genus Porphyra are distributed all over the world. Over 100 species are recognized worldwide with approximately 30 species recognized in Japan. Some of them are edible, and they are called laver or ‘nori’. The annual sales of their products are estimated at several billion US dollars. Among them, Porphyra yezoensis is the most important marine crop and has been used widely in Japan. Porphyra has a dimorphic life cycle that consists of macroscopic leafy thalli (gametophytic generation) and microscopic filamentous thalli (sporophytic generation). Some species of Porphyra have an asexual cycle consisting of repetition of leafy thalli through monospores. Porphyra is useful in the laboratory because it completes its main life cycle in laboratory culture within a few months and also because of the small number of chromosomes (2–7) in the haploid phase.1 The haploid genome sizes in Porphyra (2.7–5.3 × 10^8 bp) are of the same order of magnitude as those of Arabidopsis thaliana.2,3 A few genetic studies to date have been known in Porphyra.4–6 Also, some infrastructure arrangements for advanced research on Porphyra, including axenic tissue culture,7 strain-preservation,8 expressed sequence tag (EST) analysis,9 DNA array analysis (Kitade et al. unpubl. data, 2002) are now in progress. Porphyra yezoensis has recently received great interest as a model organism for fundamental and applied studies in marine sciences.

It is desirable to arrange a genetic map in order to generalize P. yezoensis as a model organism. Recently, genetic linkage maps based on a wide variety of new molecular markers have been constructed. In plants, most linkage maps have been constructed using randomly amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), cleaved amplified polymorphic sequences (CAPs), amplified fragment length polymorphisms (AFLPs) or simple sequence repeats (SSRs). Amplified fragment length polymorphisms analysis is known as a robust and efficient means for genetic mapping or linkage analysis in some plants.10–13 However, to date, there have been few reports on genetic markers for genome mapping in marine macroalgae. In the
present study, we detected AFLP markers with three pure lines in order to promote genetic analysis in *Porphyra yezoensis* (Bangiales, Rhodophyta).

**MATERIALS AND METHODS**

**Samples**

Leafy thalli of strains of *P. yezoensis* (TUH-25, TU-1 and TU-2) were used. TU-1 and TU-2 strains were established by Kuwano *et al.* The TUH-25 strain was derived from a clonal monospore of the H-25 strain and established in the present study by applying the slightly modified method of Kuwano *et al.* These were cultured in enriched sea life (ESL) medium (Table 1) at 15°C and 50 μmol/m² per s provided by cool-white fluorescent lamps (10 : 14 h L : D cycle) with aeration. The ancestor of TU-1 originated from the Kisarazu breeding ground (Chiba, Japan) and the ancestors of TU-2 and TUH-25 originated from the Futtus breeding ground (Chiba, Japan) close to Kisarazu. These three strains showed different color phenotypes (TU-1: wild type, TU-2: green type, TUH-25: red type; Fig. 1).

**Genomic DNA isolation**

Genomic DNAs of these three strains were isolated from each fresh leafy thallus (1 g) according to the method of Nakajima *et al.* The sample (100 mg, fresh weight) was ground in 500 μL guanidinium-based buffer (4.2 mol/L: guanidinium thiocyanate, 100 mmol/L Tris-HCl [pH 7.5], 0.05% sarcosyl) and 5 μL 2-mercaptoethanol with a mortar and pestle. After 3 min at 60°C, the supernatant was collected after centrifugation (19 000 g, 5 min, 4°C). An equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) was added to the sample and shaken gently for 15 min at room temperature. The upper phase was re-extracted two more times after centrifugation (19 000 g, 10 min). The tube was shaken after adding 0.1 volume of 3 mol/L sodium acetate and 2 volumes of –20°C 100% ethanol. It was then immediately centrifuged at 19 000 g for 3 min. The precipitate was washed by centrifugation (19 000 g, 3 min) with 1 mL of 70% ethanol and dried in a vacuum.

**Table 1** Composition of enriched sea life (ESL) medium used to culture strains of *P. yezoensis* (TUH-25, TU-1 and TU-2)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sealife powder</td>
<td>35.0 g</td>
</tr>
<tr>
<td>ESS2 stock solution</td>
<td>10 mL</td>
</tr>
<tr>
<td>pH</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*Marinetech Co., Ltd, Tokyo, Japan; *Kitade *et al.*

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**Fig. 1** Three pure lines of *Porphyra yezoensis* TU-1, TU-2 and TUH-25 (1) and dry specimens of the three pure lines (2).
The AFLP protocol developed by Vos et al.16 was similar to the AFLP analysis system II kit (Gibco, Tokyo, Japan). Genomic DNA (250 ng) was digested with 1.3 U each of EcoRI and MseI restriction enzymes. The EcoRI and MseI adapters were ligated to the ends of restricted fragments. The ligated DNA was preamplified using EcoRI and MseI directed primers. The MseI directed primer contained one selective nucleotide \((N+1)\) and the EcoRI primer contained no selective nucleotide \((N+0)\). The preamplification was performed in a total volume of 50 μL containing approximately 2.5 ng ligated DNA, 40 μL preamplification primer mixture, 5 μL of 10× polymerase chain reaction (PCR) buffer for AFLP and 1 U Taq DNA polymerase. Polymerase chain reaction for preamplification was performed as follows: one cycle of 94°C for 2 min and 20 cycles of 90°C for 30 s, 56°C for 1 min and 72°C for 1 min. The preamplification products were then diluted 50-fold with distilled water (DW). Selective amplification was carried out using five primer pair sets. One EcoRI (E-AA) and five MseI end-directed (M-CAA, M-CAC, M-CAG, M-CAT, M-CTA) primers were used. EcoRI adapter-directed primer was end-labeled using \(_{32}^{32}\text{P}\)-ATP and T4 polynucleotide kinase. The selective amplification reaction was similar to that for preamplification except that 5 μL of 50-fold diluted preamplification products was used as a template, and 1.25 μL labeled EcoRI primer and 2.25 μL unlabeled MseI primer were used. Polymerase chain reaction for selective amplification was performed as follows: one cycle of 94°C for 30 s, 65°C for 30 s, 72°C for 1 min, 12 cycles of 94°C for 30 s, 64°C for 30 s, 72°C for 1 min, 10 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 1 min. The reaction was stopped by adding 25 μL formamide loading buffer. The samples were denatured at 95°C for 5 min and then quickly cooled on ice. The PCR mixture (3 μL) was loaded onto a 6% denaturing polyacrylamide gel (1 × TBE, 7.5 M urea). Electrophoresis was performed at a constant 1600 V for 2 h. After drying, the gel was exposed to an imaging plate autoanalyzer (Mac BAS; Fuji film, Tokyo, Japan) for 1 day.

RESULTS AND DISCUSSION

The scheme of detection of AFLP is illustrated in Fig. 2. Polymerase chain reaction products were obtained in all samples examined. The same patterns of AFLP were detected in different generations of each strain (data not shown).

We could detect polymorphisms in five AFLP markers of three strains and they showed a total of 227 bands (Fig. 2). There were 50 common bands between TU-1 and TU-2 strains, 35 common bands between TU-1 and TUH-25 strains and 35 common bands between TU-2 and TUH-25 strains. There were 38 original bands in the TU-1 strain, 16 bands in the TU-2 strain and 17 bands in the TUH-25 strain. Most of the polymorphisms were detected by the primer pair of E-AA and M-CAG. This primer pair showed a total of 66 bands between three strains and, in those, there were 48 polymorphic bands.

The aforementioned results suggest that the genetic backgrounds between these three strains are quite diverse even though the culture fields of their original algae are closely located. The individual single-colored phenotypes of these three strains are convenient markers for distinction and selection of the cross-fertilization plants. Amplified fragment length polymorphisms in three strains of _Porphyra yezoensis_ detected their difference, and also confirmed clonal lines have been obtained in the present study.

We have been studying genetic analyses on various biological phenomena by applying _P. yezoensis_ TU-1 as a basic strain. We have clarified AFLP polymorphisms between strains TU-1, TU-2 and TUH-25. We have also confirmed that there are some differences in phenotype between these strains (i.e. growth, morphology and development (data in detail not shown)). TU-1 was a strain prolific with monospores, TU-2 was characterized by the slender shape of gametophytic thalli and TUH-25 was characterized by high (tall) growth and the wavy shape of a marginal portion of gametophytic thalli.

The AFLP technique, which is based on the selective PCR amplification of restriction frag-
ments from a total digest of genomic DNA, is a low-cost and simple protocol for gene mapping and linkage analysis. The amplified restriction fragments originate predominantly from unique loci on the genome and they maintain valuable sources of DNA polymorphisms. The AFLP technique is powerful, and the major application of them is as follows: marker-assisted breeding, genome analysis, molecular phylogeny, population genetics and transcript analysis. This is the first report on AFLPs of clonal lines in marine macroalgae as far as we know. The results obtained in the present study suggest that AFLP markers and clonal lines are promising tools for gene mapping in *P. yezoensis*. We anticipate that a genetic analysis of *P. yezoensis* will be promoted by applying clonal lines and the AFLP technique described here.

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REFERENCES


