THE COMPARISON OF *PORPHYRA TENERA* AND *ULVA PROLIFERA* USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

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ABSTRACT

Pyropia tenera is a red algal species in the genus *Pyropia* and *Ulva prolifera* is a species of seaweed in the family Ulvaceae. The genetic diversity of two species of seaweed were analyzed by Random amplified polymorphic DNA (RAPD). Overall, 26 and 28 fragments were generated *P. tenera* and *U. prolifera*, respectively. A total of 15 (57.7%) of these bands were polymorphic among *P. tenera*. 14 (50.0%) of these bands were polymorphic among *U. prolifera*. The OPA-02-04 band and OPA-09-05 bans were amplified for *U. prolifera*, which was absent in the three populations of *P. tenera*. The OPA-10-02 band was only amplified for *P. tenera*. These bands were exhibited the useful patterns of distinction in specific species. In general, a very low genetic diversity was observed on *U. prolifera* and genetic indices of *P. tenera* showed a slightly higher than those of *U. prolifera.* For *P. tenera*, mean number of alleles per locus (*A*) was 1.577. The effective number of alleles per locus (*A*e) was 1.373. The phenotypic frequency of each band was calculated and used in estimating genetic diversity (*H*) within species. For *U. prolifera*, the mean of *A* was 0.150 and *A*e was 1.362. The mean of *H* was 0.203 across species. The values of total genetic diversity (H_T) were 0.214 for *P. tenera* and 0.203 for *U. prolifera*. The interlocus variation of genetic diversity (H_S) was 0.168 for *P. tenera* and 0.185 for *U. prolifera*. On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) was 0.213 for *P. tenera* and 0.090 for *U. prolifera.*

Keywords: Genetic variation, *Pyropia tenera*, RAPD, *Ulva prolifera.*

INTRODUCTION

Molecular biology enables easy analysis of the genetic structure of wild populations, including studies of genetic variation and gene flow among populations and of genetic differentiation between species and continental close relatives (Oiki et al., 2001).

Random amplified polymorphic DNA (RAPD) markers represent amplification products from a polymerase chain reaction (PCR) utilizing arbitrary primers and genomic DNA (Williams et al., 1990). The PCR-based RAPD technique is an attractive complement to conventional DNA fingerprinting in taxonomy. RAPD analysis appears to offer a cost- and time-effective alternative to restriction fragment-length polymorphism (RFLP) analysis. RAPD analysis is unexpectedly simple. The amplification protocol differs from the standard PCR conditions (Erlich, 1989) in that only a single random oligonucleotide primer is employed and no prior knowledge of the genome subjected to analysis is required (Hadrys et al., 1992).
Genomic DNA are able to differentiate between genetically distinct individuals or morphologically similar species, although not necessarily in a reproducible way. These

problems may be overcome if care is taken to ensure consistent reaction conditions during amplification (Bustos et al., 1998).

Pyropia tenera, also known as gim or nori, is a red algal species in the genus *Pyropia*. About 150 species of *Porphyra* are found in the worldwide (AlgaeBase), of which about 20 grow on the coast of Korea and Japan. Two of these, *P. yezoensis* and *P. tenera*, are mostly cultivated by the Korea and Japan. *Ulva prolifera* is a species of seaweed in the family Ulvaceae that can be found worldwide such as Europe, Africa, America, and Asia. This alga in Korea is virtually been a steady increase in the demand for various uses in the food. Although a species level identification of *P. tenera* and *U. prolifera* using only morphological characteristics is possible, it is often difficult to distinguish between the two species. Since the demand for seaweed is constantly on rise, their identification is of primary importance. Lot of taxonomic work has been done in early 60-70s on the morphological and anatomical features (Prasad et al., 2009). RAPD technique was used to characterize species of *Porphyra* from the western North Atlantic and adjacent Gulf of Mexico (Dutcher & Kapraun, 1994).
The objectives of the present study were 1) to investigate the level of RAPD variation in three natural populations of *P. tenera* and *U. prolifera* in southeast of Korea, 2) to quantify the genetic diversity within and between natural both species (such information can also serve as a guide to preserving the genetic resources of this ecologically important species), and 3) to produce diagnostic markers for analyzing morphologically similar species.

METHODOLOGY

Sample procedure and DNA extraction

All of the three populations of *P. tenera* and *U. prolifera* were collected from natural populations in Korea. The species sampled for this study are listed Table 1. To analyze the proportion of genetic diversity among and within populations or species, all samples were taken from two remote wild populations for each other. Twenty plants were randomly collected from each population.

Total DNA was extracted using the plant DNA Zol Kit (Life Technologies Inc., Grand Island, New York, U.S.A.) according to the manufacturer's protocol. The DNA concentration of each sample was determined spectrometrically and was electrophoresed on a 1.5 % agarose gel to confirm quality.

Table 1. Codes and geographic locations of three *Pyropia tenera* and *Ulva prolifera* populations

Codes	Localities
JUK	Jukrim-ri, Georyu-meon, Tongyeong-si, Gyeongsangnam-do
HWA	Hwadang-ri, Georyu-meon, Goseng-gun, Gyeongsangnam-do
YAN	Yang-a-ri, Sangju-myeon, Namhae-gun, Gyeongsangnam-do

RAPD analysis

Ten decamer oligonucleotides for PCR (polymerase chain reaction) were purchased from BIONICS Inc. (Seoul, Korea). All the reactions were repeated twice and only reproducible bands were scored for analyses. From 20 decamer primers (OPA-01~OPA-20) used for a preliminary RAPD analysis.

The template for PCR amplification consisted of 20 ng genomic DNA. The PCR amplifications were performed in a reaction volume of 12.5 µl containing 20 ng of template DNA, 5.0 pM primer into the *AccuPower® Taq* PCR PreMix (TaKaRa Co, Japan), and

distilled water. PCRs were performed twice for reproducibility in an TaKaRa PCR Thermal Cycler (Model: TP-600, Japan). In addition, replicate accessions were assayed in separate experiments to verify repeatability of results. The amplification products were separated by electrophoresis on 1.5% agarose gels. A 100 bp ladder DNA marker (Pharmacia) was used in the end of for the estimation of fragment size. Visualization of the nucleic acid-dye complex by UV illumination photographed under UV light using Alpha Image TM (Alpha Innotech Co., U.S.A.).

Data analysis

All RAPD bands were scored by eye and only unambiguously scored bands were used in the analyses. Because RAPDs are dominant markers, they were assumed that each band corresponded to a single character with two alleles, presence (1) and absence (0) of the band, respectively. For the RAPD resolved in more than one zone of activity, the most anodal migrating band was designated as '1', and other subsequent fragments were sequentially numbered. The percentage of polymorphic loci (P_p for population level and P_s for species level), mean numbers of alleles per locus (*A*), effective number of alleles per locus (A_e), gene diversity (*H*) (Nei, 1973), Shannon's phonetic diversity (*I*) were computed with POPGENE 1.31 (Yeh et al., 1999). The phenotype frequency of each band was calculated and used in estimating total diversity(H_T), genetic diversity within populations (H_S) proportion of total genetic diversity partitioned among populations (G_{ST}), and gene flow (*Nm*) (Nei, 1973). A phenetic relationship was constructed by the neighbor joining (NJ) method (Saitou & Nei, 1987) using the MEGA version 6.06 (Tamura et al., 2013).

RESULTS AND DISCUSSION

From the 20 decamer primers (OPA-01~OPA-20) used for a preliminary RAPD analysis, seven primers of them produced good amplification products both in quality and. Overall, 26 and 28 fragments were generated *P. tenera* and *U. prolifera*, respectively. A total of 15 (57.7%) of these bands were polymorphic among *P. tenera* (Table 2). 14 (50.0%) of these bands were polymorphic among *U. prolifera* (Table 3). The remaining fragments were monomorphic in all taxa. The number of bands for each primer varied from one to seven with an average of 3.7 fragments per primer. The size of the amplified products ranged from 300 to 2100 bp.

The OPA-02-04 band and OPA-09-05 bans were amplified for *U. prolifera*, which was absent in the three populations of *P. tenera* (Fig. 1). The OPA-10-02 band was only amplified for *P. tenera*. These bands were exhibited the useful patterns of distinction in specific species. All but one pair of genotypes differed for at least three bands and all but three pairs of genotypes differed for at least six polymorphic RAPD bands. These results indicate that RAPD marker variation can be easily detected within and among two algae species and indicates that the three wild populations used in this study should be considered genetically heterogeneous populations, with respect to RAPD markers.

Genetic diversity parameters were calculated over all populations of two species and for *P. tenera* and *U. prolifera* seperative. In a simple measure of intrapopulation variability by the percentage of polymorphic bands, YAN population showed the highest (50.0%) among *P. tenera* (Table 3). JUK population exhibited the lowest variation (38.7%). For *P. tenera*, mean number of alleles per locus (*A*) ranged from 1.385 to 1.500 with a mean of 1.577. The effective number of alleles per locus (*A*e) ranged from 1.229 to 1.317 with a mean of 1.373. The phenotypic frequency of each band was calculated and used in estimating genetic diversity (*H*) within species. As the typical populations were isolated, and patchily distributed

for aquaculture sea, they maintained a low level of genetic diversity for seventeen primers. The mean of *H* was 0.215 across species, varying from 0.133 to 0.188. In particular, YAN population had high phonetic diversity (*I*) (0.275). Interspecies diversity of *P. tenera* accounted for 33% of the total genetic diversity using isozyme analysis (Huh et al., 2006). The proportion of polymorphic loci was 0.333 and heterozygosity (*H*) over 12 loci varied from 0.100 to 0.141 with a mean of 0.127 using isozyme analyses (Fusio et al., 1985). In this study, the mean of *H* was 0.215 across species, varying from 0.133 to 0.188. Although there is not a significant difference, diversity indices of RAPD analysis were higher those of isozyme. diversity indices of RAPD analysis were higher those of isozyme. RAPD markers are able to disclose a much higher level of polymorphism than revealed by isozymes, essentially at the infraspecific level (Sonnante et al., 1997).

For *U. prolifera*, the mean of *A* was 0.150 and *A*e was 1.362. The mean of *H* was 0.203 across species. The values of total genetic diversity (H_T) were 0.214 for *P. tenera* and 0.203 for *U. prolifera* (Table 4). The interlocus variation of genetic diversity (H_S) was 0.168 for *P. tenera* and 0.185 for *U. prolifera*. On a per locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) was 0.213 for *P. tenera* and 0.090 for *U. prolifera*, indicating that 79% and 91% of the total variation were found among *P. tenera* and *U. prolifera* populations, respectively. The *Nm* for *P. tenera* was estimated to be low (1.842). However, the *Nm* for *U. prolifera* was estimated to be high (5.060). In general, a very low genetic diversity was observed on *U. prolifera* and genetic indices of *P. tenera* showed a slightly higher than those of *U. prolifera.*

Genetic identity (*I*) based on the proportion of shared fragments was used to evaluate relatedness among populations. Clustering of populations, using the NJ algorithm, was performed based on the matrix of calculated distances (Fig. 2). The tree showed genetic differentiation among Korean *P. tenera* and *U. prolifera*. The phylogenic tree showed two or three distinct clades.

Table 2. Measures of genetic variation for *Porphyra tenera*. The number of polymorphic loci (*N*p), percentage of polymorphism (*P*p), mean number of alleles per locus (*A*), effective number of alleles per locus (A_E), gene diversity (*H*), and Shannon's information index (*I*)

Population	*N*p	*P*p	*A*	A_E	*H*	*I*
JUK	10	38.7	1.385	1.229	0.133	0.199
HWA	12	46.2	1.462	1.335	0.188	0.273
YAN	13	50.0	1.500	1.317	0.185	0.275
Total	15	57.7	1.577	1.373	0.215	0.318

Table 3. Measures of genetic variation for *Ulva prolifera*. The number of polymorphic loci (*N*p), percentage of polymorphism (*P*p), mean number of alleles per locus (*A*), effective number of alleles per locus (A_E), gene diversity (*H*), and Shannon's information index (*I*)

Population	*N*p	*P*p	*A*	A_E	*H*	*I*
JUK	13	46.4	1.464	1.291	0.166	0.248
HWA	13	46.4	1.464	1.333	0.190	0.277
YAN	14	50.0	1.500	1.349	0.198	0.291
Total	14	50.0	1.500	1.362	0.203	0.296

Table 4. Estimates of genetic diversity of *Pyropia tenera* and *Ulva prolifera*. Total genetic diversity (H_T), genetic diversity within populations (H_S) proportion of total genetic diversity partitioned among populations (G_{ST}), and gene flow (*Nm*)

Species	H_T (SD)	H_S (SD)	G_{ST}	*Nm*
P. tenera	0.214(0.043)	0.168(0.034	0.213	1.842
U. prolifera	0.203(0.047)	0.185(0.040)	0.090	5.060

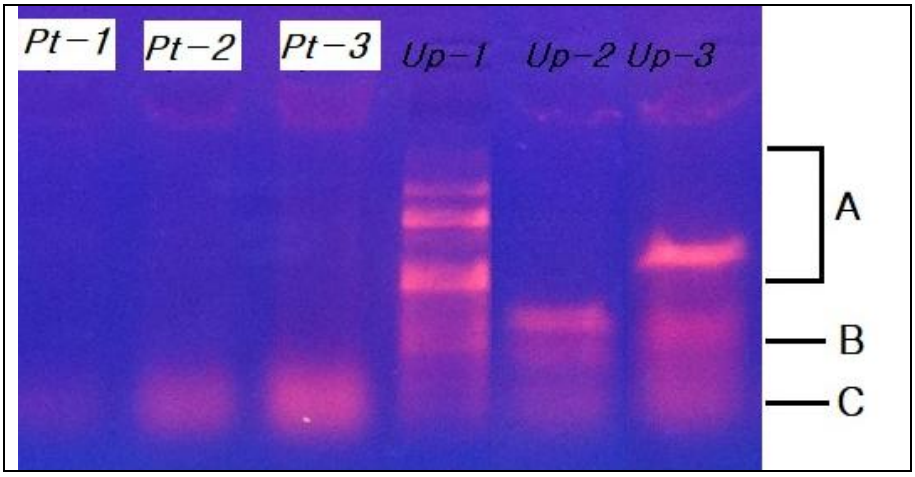

Fig. 1. The profiles of *Porphyra tenera* (*Pt*-1 ~ *Pt*-3) and *Ulva prolifera* (*Up*-1 ~ *Up*-3) using Operon primer OPA-9. Bands of region A was polymorphic bands in *U. prolifera*. B was specific band for *U. prolifera.* C was common band in both species.

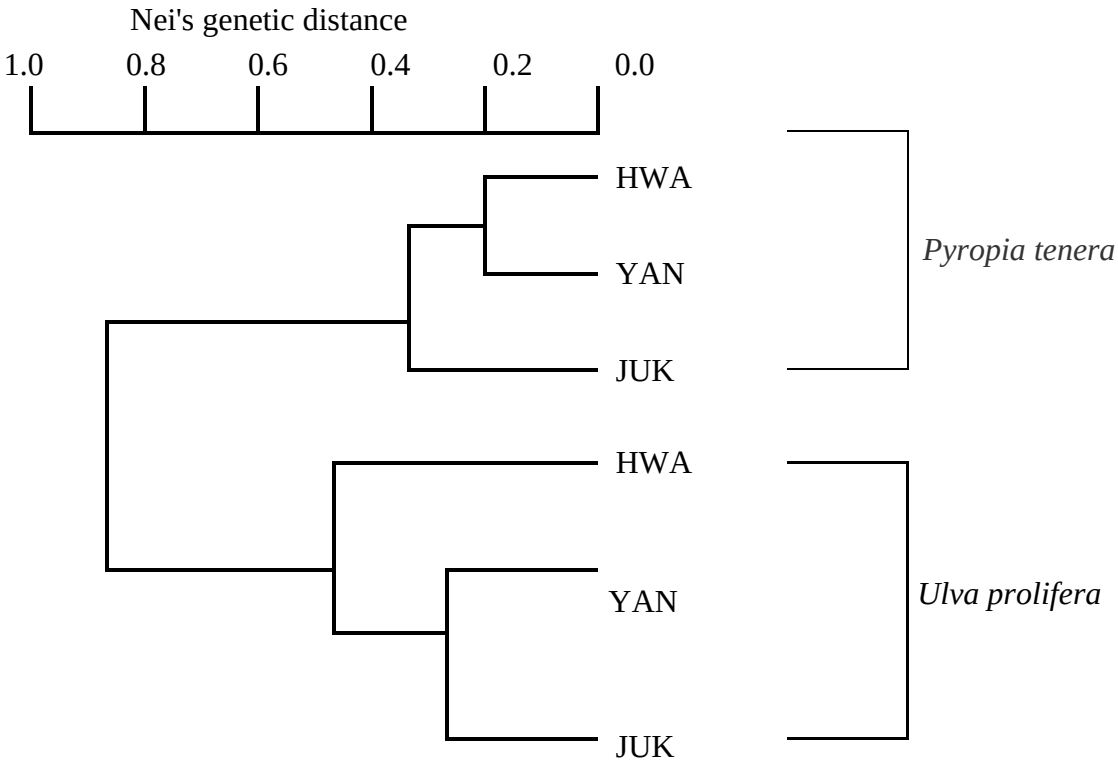

Fig. 2. A phenogram showing the relationships among three populations of *Pyropia tenera* and *Ulva prolifera* based on data of genetic distance obtained by RAPD.

CONCLUSIONS

Overall, the results demonstrate the utility of the RAPD technique for evaluating genetic relationships and contrasting levels of genetic diversity among populations of *Pyropia tenera* and *Ulva prolifera.*

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