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Morphological and Random Amplified Polymorphic DNA features in populations of *Ceramium kondoi* (Rhodophyta)

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Morphological and RAPD features of *Ceramium kondoi* populations were investigated and compared in different locations in Korea. The plant length and branching pattern were more variable in Jindo population than others. RAPD data showed that *C. kondoi* plants were divided into two clades; the southern group including Jindo and Bangpo population, and the northern group including Yonpyongdo and Oeyondo population. Morphological features in *C. kondoi* populations corresponded with RAPD data, which differed from those of *C. boydenii* from the same location. These results suggest that RAPD might be useful for elucidating genetic variation among the wild populations of *C. kondoi*.

Keywords: *Ceramium kondoi*, distribution, morphologies, RAPD, Rhodophyta

Ceramium kondoi Yendo is a ceramiaceous red alga and has been distinguished from the other species of the genus *Ceramium* by the trichotomous ramification and thicker cortex (Yendo, 1920; Nakamura, 1965). The species is often confused with *C. rubrum* (Hudson) Agardh, a well-known cosmopolitan species in the algae (Dixon, 1960). For example, Nakamura (1950) reported that some European specimens identified as *C. rubrum* might be named as *C. kondoi* because of having trichotomous branches, while some Japanese specimens identified as *C. kondoi* rather be given the name of *C. rubrum* because of having dichotomous branches. Until now *C. kondoi* has been reported to occur in the northeast Pacific (Boo and Lee, 1994; Yoshida *et al.*, 1995) whereas *C. rubrum* occurs in the world except the above area. On the other hand, Garbary *et al.* (1980) reported that a large number of trichotomous branches were produced in gametophytic and tetrasporic plants in a Canadian *C. rubrum* population, without mentioning taxonomic value of trichotomous branches in this species. On the basis of the year-round observation of branching pattern of *C. kondoi* from Korea, Boo and Yoon (1993) considered the trichotomous branching as a taxonomically valid feature.

Ceramium kondoi plants occur in the intertidal area on the western and the southern coast of Korea, where they are under the influence of mud or fresh water. They appear young in late winter, mature around the late spring and then disappear in autumn, that shows the seasonal change of life history phases in Korean waters (Boo and Yoon, 1993). The species shows extreme infraspecific variation in cortex thickness, branching pattern, and tetrasporangial arrangement, and it has been separated into the four subspecific forms (Nakamura, 1965). These features also show variations in culture (Suh and Lee, 1984), but there have been no reports on morphological variation in different locations in the field.

Random amplified polymorphic DNA (RAPD) has been known as a rapid and simple method to study DNA polymorphism using polymerase chain reaction (PCR) with arbitrary primers (Williams *et al.*, 1990). RAPD has already been shown to provide useful data for strain, population, and taxonomic sorting in a wide variety of organisms using different primers (Heusden and Bachmann, 1992; Lifante and Aguinagalde, 1996), but methodological noise of RAPD should be removed by careful and reproducible experiments (van Oppen *et al.*, 1996). Although most of RAPD approaches use cultured strains in algae (Patwary *et al.*, 1993; van Oppen *et al.*, 1996), RAPD-PCR has been applied to assess the genetic variation of different populations using the wild populations (Ho *et al.*, 1995).

To elucidate the local variation of *C. kondoi*, morphological observations and RADP analyses were given

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Table 1. Materials used for morphological and RAPD studies

Species	Population code	Location	Collection Date	
			Morphologies	RAPDs
Ceramium kondoi	YP	Yonpyongdo	VIII 10, 1993	III 2, 1994
	DJ	Dukjeokdo	-	VIII 3, 1993
	BP	Bangpo	V 19, 1993	V 28, 1994
	OY	Oeyondo	V 6, 1993	VI 12, 1994
	KP	Kyokpo	IV 25, 1993	V 27, 1994
	JD	Jindo	IV 3, 1993	IV 24, 1994
	DS	Dolsando	IV 4, 1993	-
C. boydenii	*C. bo*	Bangpo	-	V 28, 1994

to the six different populations in Korea. All of these data are discussed with implications for taxonomy and distribution of *C. kondoi.*

MATERIALS AND METHODS

Materials

As seen in Table 1, *Ceramium kondoi* plants were collected on the west and the south coast of Korea for comparing morphological and RAPD data. They occurred in the tide pools and on rocky or small pebbles in the littoral areas. Above 25 plants were randomly collected and kept in the 5% neutral formalin-seawater solution. *C. boydenii* Gepp was used as an outgroup taxon in RAPD because of having similar branching pattern with *C. kondoi.*

To extract DNA, field materials were carried on ice to laboratory and washed with filtered seawater. Sand, mud and epiphytes were cleaned with brush and washed in sterile distilled water. The apical branches were cut and stored in the deep freezer of -70°C.

Morphological observation

Each plant was examined for reproductive organs in every collection. Plant length was measured and compared in the different sites and in the different life history phases. The number of di-, tri- or tetrachotomous branches was counted as percentage of total number of branches on a main axis.

Extraction and purification of genomic DNA

Samples of about 2 g were pulverized to a powder in liquid nitrogen with pestle and mortar. The algal powder was immediately mixed into 10 ml cetyltrimethylammonium bromide (CTAB) buffer and was incubated at 60°C for 1 hr with occasional stirrings. Extraction and purification of genomic DNA followed a few modifications of Fang *et al.* (1992). The purified DNA was dissolved in sterilized distilled water.

Table 2. Base sequence of useful random primers and number of amplified products in *C. kondoi* plants

Primer name	Nucleotide sequence (5' to 3')	Number of amplified products
A-02	TGC CGA GCT G	12
A-04	AAT CGG GCT G	10
A-10	GTG ATC GCA G	6
A-11	CAA TCG CCG T	5
A-17	GAC CGC TTG T	10

RAPD amplification

The polymerase chain reaction (PCR) was performed in a volumne of 25 ul containing 10x buffer, 0.2 mol/L of each dNTP, 0.5 unit primer, 5 ng template DNA and 0.5 unit *Taq* DNA polymerase (Promega). The RAPD primer kit was obtained from Operon Technologies Inc. (Almeda California, USA). Sequences and sources of primers giving informative amplification are listed in Table 2.

Amplification was carried out in a FTC 2000 capillary thermal cycler (Dachan Medical Company, Seoul) programmed as follows: 94°C (5 min) for the initial separation of double strand, then 45 cycles of 94°C (50 s) for denaturation, 36°C (50 s) for annealing, and 72°C (2 min) for extension. Amplified products were separated on 1.5% agarose gel by electrophoresis in 1 X TBE and detected by staining with ethidium bromide. The gels were photographed under UV light with Polaroid film 667. DNA marker λ/*Hind*III for knowing the molecular weight of the PCR products was used as a standard.

Data analysis

RAPD data were obtained with 5 arbitrary primers. The genetic polymorphism was analyzed based on PCR products. Bands were scored as presence or absence and band-sharing analysis was conducted us-

ing phylogenetic analysis using parsimony (PAUP) (Swofford, 1990).

RESULTS

Morphologies

Ceramium kondoi plants grew vigorously along the coast of the Yellow sea and the south coast of Korea, but rarely along the east coast. The plants grew on the small pebbles and in the tidepools in the intertidal area. They were usually aggregated into a large tuft. Reproductive organs were found in most of plants collected. Each life history phase was seen in the similar ratio in Oeyondo, Kyokpo and Jindo populations while about 70% plants had tetrasporangia in Yonpyongdo and Bangpo population. In Jindo population, the ratio of the banded to the scattered arrangement of tetrasporangia was observed 1 : 1. The ratio was very high in Bangpo and Oeyondo whereas low in Dolsando population.

The mean length of *C. kondoi* plants differed among the populations (Fig. 2). The maximum length of 15.1 cm was found in Jindo, but with the highest standard deviation of 5.4. The minimum length was observed 7.3±2.1 cm in Yonpyongdo, while the mean length of all plants measured in this study was 12.2± 3.4 cm.

Branches of *C. kondoi* were produced pseudodichotomously by oblique division of apical cells, so the branching pattern seemed dichotomous, trichotomous and tetrachotomous. The mean occurrence of dichotomous branches on a single main axis was 73.7±16.3% in all local plants. The maximum occurrence of dichotomous branches was 96.9±5.6% in Yonpyongdo, and the minimum 53.3±21.4% in Bangpo (Fig. 2). The frequency of trichotomous branches was the maximum of 45±20.4% in Bangpo. It was also higher in Jindo and Kyokpo population than the mean frequency from all populations. The tetrachotomous branches were produced high in Jindo population than others.

RAPD

A total of 20 random primers were screened for amplification of the genomic DNA from the six local populations of *C. kondoi*, but 15 primers showed

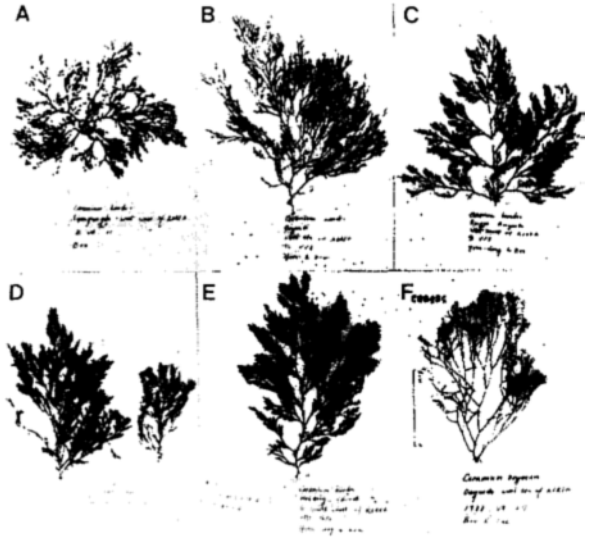

Fig. 1. *Ceramium kondoi* plants from Yonpyongdo (A), Oeyondo (B), Bangpo (C), Kyokpo (D), Jindo (E) and *C. boydenii* (F) on the western coast of Korea.

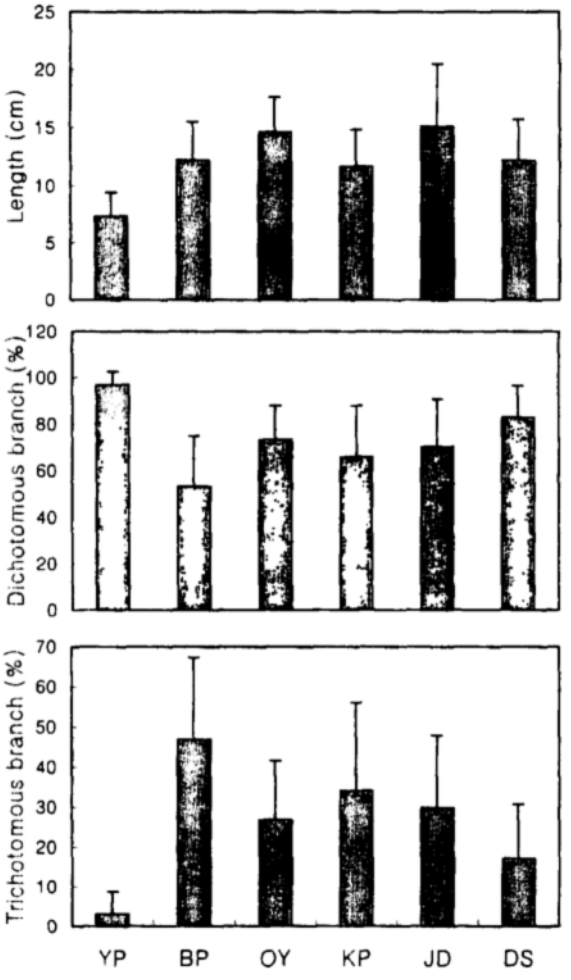

Fig. 2. Variation in mean plant length and percentage of occurrence of dichotomous and trichotomous branches of *Ceramium kondoi* plants in different locations. The abbreviations are in Table 1.

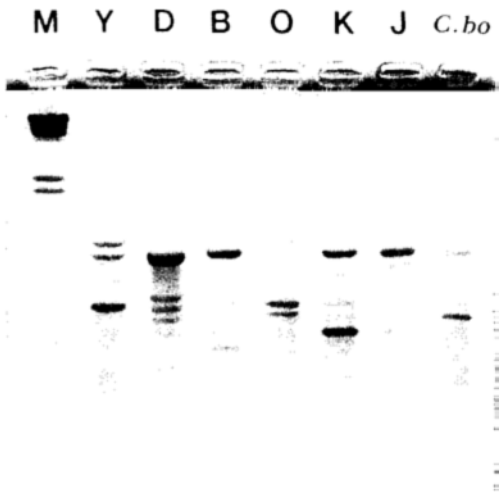

Fig. 3. Amplified products of genomic DNA of *Ceramium kondoi* and *C. boydenii* plant with A-04 primer. Size markers (λ/*Hind*III) are on the left side. Population codes are same in Table 1.

fuzzy and complex patterns of bands that proved difficult to interpret.

PCR below 35°C of annealing temperature produced 10 or more amplification products while PCR over 38°C few products. The reproducibility of amplified products made certain with the 5 pair primers of decamer listed in Table 2. The amplification products using the A-04 primer were shown in Fig. 3. All amplification products obtained in this study were graphed as a bandmap (Fig. 4). The bandmap showed the tendency of grouping local populations based on the shared bands.

It was possible to score 43 band positions from *C. kondoi* plants. One band was shared both in *C. kondoi* and *C. boydenii*, and two bands including the above were shared among all populations of *C. kondoi*. Although 24 bands were present or absent in a single population, the remaining 17 positions were shared more than 2 bands in at least two populations, which were used for making trees.

Parsimony analysis of RAPD data produced a genetic tree with the minimal length. The tree divided *C. kondoi* populations into two groups (Fig. 5): the first group of Jindo and Bangpo population and the second of Oeyondo and Yonpyongdo population. *C. boydenii* was shown to be different from *C. kondoi* populations.

DISCUSSION

Temporal change of *Ceramium kondoi* plants in the field has been shown to have an obvious seasonality in a single location on the western coast of Korea (Boo and Yoon, 1993), but this may be the first case story of spatial changes in morphological variation. This study of *C. kondoi* agrees with the

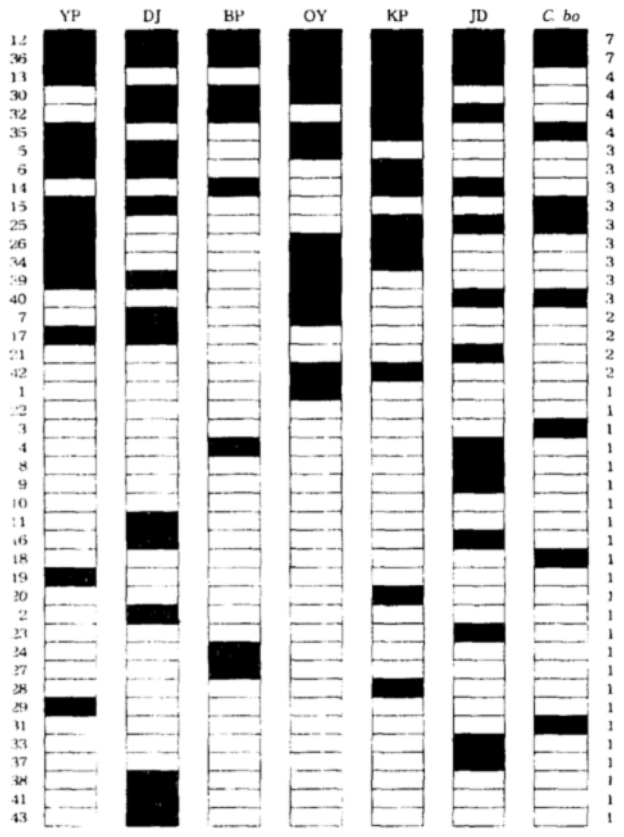

Fig. 4. Bandmap of shared and polymorphic amplified products (hatched rectangle) in *Ceramium kondoi* plants. Population code is at the top of each column as listed in Table 1, and genotype code in the right margin, and the number of amplified products in the left.

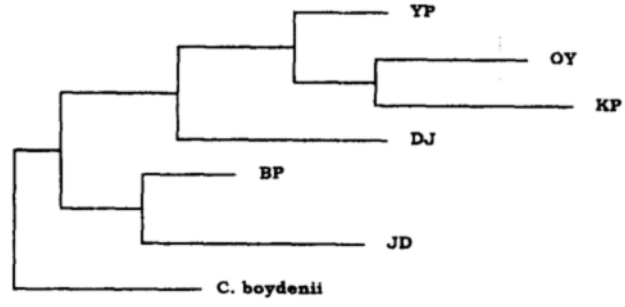

Fig. 5. A single most parsimonious tree based on RAPD data of *Ceramium kondoi* populations. Length=57 steps, CI=0.684, RI=0.419, and RCI=0.287. *C. boydenii* was used as an outgroup.

case that the life history phases of the ceramiaceous red algae depend on the environmental factors like temperature and irradiance and then are shown to be in different ratios in different local populations (Boo and Lee, 1994). The fact that Jindo, Kyogpo and Oeyondo plants had the similar ratio of life history phases suggest that the populations might be in young stage of life cycle. This observation corresponds with the tetrasporangial features in that most of tetrasporangia were arranged in banded form in the above population whereas in scattered form in Yonpyongdo and Bangpo populations. However, Nakamura (1965) used the tetrasporangial arrangement as a diagnostic feature for separating infraspecific taxa.

Branching pattern of *C. kondoi* seems to be variable in different populations. The variability of branching pattern is shown high in Jindo population, judging from the high occurrence of trichotomous and tetrachotomous branches. The trichotomous branches are reported to occur in a frequency of 20-30% in Oeyondo island throughout the year (Boo and Yoon, 1993), that agree with this study showing an occurrence frequency of 20-40% in all populations. Japanese plants from Harius in Hokkaido, the type locality of *C. kondoi*, also have a similar frequency of trichotomous branches (unpublished data). Garbary *et al.* (1980) observed that *C. rubrum* plants from Canada had a large number of trichotomous branches. In this respect, *C. kondoi* plants from Korea and Japan are similar with the Canadian *C. rubrum* plants. The biogeographical discussion of *C. kondoi* have been given by Boo and Yoon (1993), who reported that *C. kondoi* in the northeast Pacific seems a vicariant species to *C. rubrum* in the northwest Pacific. But the query of *C. kondoi* on species limitation and geographical distribution needs a further world scale study.

Polyphenols, pigments, secondary metabolites and polysaccharides disturb extraction of DNA from algal cells (Hong *et al.*, 1992). To get clean and enough quantity of genomic DNA, lots of methods have been developed (e.g. Saunders, 1993), but CTAB extraction of genomic DNA from *C. kondoi* made our RAPD-PCR easy and rapid.

Technical limitations and considerations of RAPD have been discussed in details (Hadrys *et al.*, 1992; Clark and Lanigan, 1993) and the application of RAPD for algal taxonomy have increased with time (van Oppen *et al.*, 1996). RAPD using cultured materials have revealed the genetic variation more efficiently (Patewary *et al.*, 1993), but wild ones could be used for finding genetic similarity among geographical populations in the *Sargassum* species of the brown algae (Ho *et al.*, 1995). We also used wild plants of *C. kondoi* for RAPD because of the study on genetic variation of local populations in different environments. There are no reports of RAPD on the *Ceramium* species from the northeast Pacific.

Biogeographical clustering of *C. kondoi* populations is clear as seen in Fig. 6. Although all populations are in a distance of about 320 km on the western coast of Korea, the clades by PAUP show the geographical reaches among populations. The cluster of Jindo and Bangpo population show a tendency of southern and inshore characteristics, the other cluster northern and open sea characteristics. The one exception is the population of Dukjeokdo which is under the big influence of fresh water and mud from Hangang river. The fact that RAPD data correspond with the geographical distance has been reported in the algae as well as the grasses (van Oppen *et al.*, 1996; Heusden and Bachmann, 1992).

The good systematic approaches need to combine many available data like morphologies, RAPD and others from the possible experimental techniques (Halward *et al.*, 1993; Heusden and Bachmann, 1992), but it is very difficult for morphological data to correlate with genetic data. Here we just aimed to know the tendency of morphological and genetic variation in different local populations. Morphological data in *C. kondoi* populations seem to correspond with RAPD data, which differ from those of *C. boydenii* from the same location. These results suggest that RAPD might be useful for genetic variation among the wild populations of *C. kondoi*.

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