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Restriction Enzyme Analysis of DNA from Conchocelis of *Porphyra* Species

ABO Kouji and EGOSHI Kazuo

ノリ糸状体DNAの制限酵素による分析

安保康治・江越和夫

ノリの種を識別する方法を確立する目的で，アガロース電気泳動法により，14種の制限酵素によるDNAの消化性の有無を比較した。この実験に用いたノリの糸状体は，ミノミアサクサノリ，アサクサノリ松川，ナラワスサビノリ，スサビノリ佐賀10号，スサビノリ緑芽，島原イチマツノリであった。全種のDNAとも，*Hind*Ⅲ，*Kpn*Ⅰ，*Hha*Ⅰ，*Dra*Ⅰ，*Xba*Ⅰでは消化されず，*Hae*Ⅲ，*Alu*Ⅰ，*Sal*Ⅰでは消化された。しかし，*Bam*HⅠ，*Pst*Ⅰ，*Eco*RⅠ，*Xho*Ⅰ，*Msp*Ⅰ，*Apa*Ⅰの6種類の制限酵素では，6種間に消化性の差異があり，これらによって種の識別が可能であった。以上より，ノリの種同定には，6種類の制限酵素によるDNAの消化性が有効であろうと思われた。

キーワード：アマノリ，糸状体，DNA，制限酵素，電気泳動，同定
Key words : *Porphyra*, conchocelis, DNA, restriction enzyme, electrophoresis, identification

Twenty to thirty *Porphyra* species are said to be in cultivation. They comprise *P. tenera*, *P. yezoensis* and *P. yezoensis* f. *narawaensis*, which in turn are divided into many local selected species. These are distinguishable by the morphological features of the thallus, namely, shape, size, color, and thickness. However, these morphological characteristics are not clear because *Porphyra* has simple structures that is strongly influenced by environmental changes.

Previously, using conchocelis (diploid) cultivated under constant conditions as samples for six typical species of *Porphyra*, we examined the isozymes of fifteen enzymes by polyacrylamide gel electrophoresis[1,2]. The six species could be distinguished by the isozyme profiles obtained.

Recently, in higher plants, DNA analysis by means of restriction enzymes has often been used to biochemically classify and identify species[3-5]. But, only a few reports have been published about *Porphyra* DNA[6]. Previously, we reported a method of DNA isolation from *Porphyra* for comparison of differences in nucleotide sequences[7].

In this study, using conchocelis as samples for six typical species of *Porphyra*, we examined the DNA digestion with fourteen restriction enzymes by agarose gel electrophoresis. The six species could be distinguished from the characteristic DNA profiles obtained on several enzymes.

Materials and Methods

Samples

Porphyra conchocelis was a gift from Saga Prefectural Ariake Fisheries Research and Development Center in June 1992. The samples were then cultivated at 18±0.5℃ under 12L : 12D at 16.7μ mol m^{-2} s^{-1} in modified SWM-Ⅲ medium[8] which had been filtered and sterilized. The *Porphyra* conchocelis examined consisted of *P. tenera* (Minomi), *P. tenera* (Matsukawa), *P. yezoensis* f. *narawaensis*, *P. yezoensis* (Saga10), *P. yezoensis* (Green), and *P. seriata* (Shimabara).

DNA isolation

The DNA was isolated from *Porphyra* conchocelis as reported previously[7], according to the method of Zao[9].

Namely, Sample, 1−2 g surface-dried tissue, was ground and mixed with 1×SSC (Nippon Gene Inc.), proteinase K (Wako Inc.), and SDS (Wako Inc.). The mixture was incubated at 65℃ for 30 min. Then, phenol extraction and chloroform extraction were repeated twice. Then the ethanol precipitation was dissolved in TE buffer (Nippon Gene Inc.) and RNase (Nippon Gene Inc.) was added. The mixture was incubated at 37℃ for 2 hr. Phenol extraction and chloroform extraction were repeated twice. The final ethanol precipitation was dissolved in 1 ml TE buffer and stored at −20℃.

Restriction enzyme digestion and electrophoresis

The DNA was digested with various restriction enzymes, *Hind*Ⅲ, *Kpn* Ⅰ, *Hha* Ⅰ, *Dra* Ⅰ, *Xba* Ⅰ, *Hae*Ⅲ, *Alu* Ⅰ, *Sal* Ⅰ, *Bam*H Ⅰ, *Pst* Ⅰ, *Eco*R Ⅰ, *Xho* Ⅰ, *Msp* Ⅰ, and *Apa* Ⅰ (Nippon Gene Inc.) according to the protocol suggested by the supplier.

The DNA, the reaction products and DNA MW Marker 1 (λ DNA/*Hind*Ⅲ digestion, Nippon Gene Inc.) were separated in 1% agarose gel (Agarose ME, Wako Inc.) at 6 Volt/cm for 2 hr. The gel contained ethidium bromide (5μ g/ml) and was prepared with TAE buffer (40 mM Tris-acetate, pH 8.3, 1 mM EDTA).

DNA quantitation and qualitation[10]

The DNA concentration was determined by uv absorption, assuming that 1 unit of absorbance at 260 nm equals 50μ g of DNA/ml. The ratio of A_{260}/A_{280} was used for estimating protein contamination.

Results and Discussion

From six species of *Porphyra* conchocelis, DNA was isolated as reported previously[7]. Table 1 shows their properties. We obtained 4.1−5.5 mg of DNA from 1.4−1.7 g of conchocelis. The higher yield depended on diploid tissue and percentage of cells which had been disrupted during homogenization. To determine the degree of protein contamination, the absorbance (A) of isolated DNA solutions was examined in the uv range. The ratio between readings at

260 and 280 nm (A_{260}/A_{280}) is about 1.80 for the standard DNA solution. The value is shifted by the presence of proteins in the DNA solution[10]. The values for these DNA were the range, 1.64−1.77 being very close to 1.80, indicating that there was little protein contamination in these DNA preparations.

Table 1 Properties of DNA isolated from *Porphyra* conchocelis.

	P. tenera (Minomi)	*P. tenera* (Matsukawa)	*P. yezoensis* f. *narawaensis*	*P. yezoensis* (Saga10)	*P. yezoensis* (Green)	*P. seriata* (Shimabara)
Sample weight (g)	1.5	1.6	1.5	1.4	1.7	1.6
DNA solution volume (ml)	1.0	1.0	1.0	1.0	1.0	1.0
OD at 260 nm	82	90	95	98	92	110
OD at 280 nm	48	55	54	57	52	65
A_{260}/A_{280}	1.70	1.64	1.76	1.72	1.77	1.69
DNA concentration (mg/ml)	4.1	4.5	4.8	4.9	4.6	5.5

These preparations were analyzed by agarose gel electrophoresis (Fig.1). All the preparations isolated from six species exhibited a single band on the agarose gel, suggesting that these DNAs were electrophoretically homogeneous. Their sizes were determined to be approximately 20 kbp in common using the DNA marker.

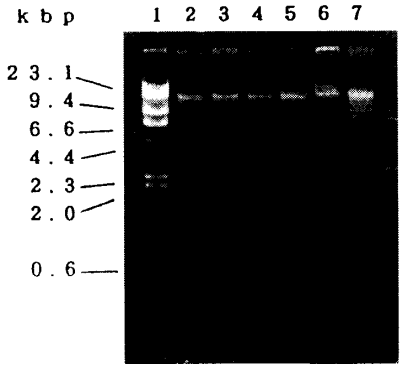

Fig. 1 Electrophoretic patterns of DNAs from different species of Porphyra conchocelis. Lane1 ; *Hind*Ⅲ-digested λ DNA, Lane2 ; *P. tenera* (Minomi), Lane3 ; *P. tenera* (Matsukawa), Lane4 ; *P. yezoensis* f. *narawaensis*, Lane5 ; *P. yezoensis* (Saga10), Lane6 ; *P. yezoensis* (Green), Lane7 ; *P. seriata* (Shimabara)

Next, the DNAs were digested with 14 restriction enzymes. Table 2 shows the restriction enzymes used in this experiment and respective base sequences on the recognition sites. None of the DNAs from the species were digestible with *Hind*Ⅲ, indicating that the sequence, AAGCTT is not present in any of the DNAs. The same results were obtained with the enzymes, *Kpn* Ⅰ, *Hha* Ⅰ, *Dra* Ⅰ, and *Xba* Ⅰ. However, *Hae*Ⅲ digested all the DNAs. Therefore, the sequence, GGCC was thought to exist in them. In addition, *Alu* Ⅰ and *Sal* Ⅰ exhibited the same property as *Hae*Ⅲ.

Table 2 Summary of characteristics of restriction enzymes.

Endonucleases	Recognition specificities
*Hind*Ⅲ	AAGCTT
Kpn I	GGTACC
Hha I	GCGC
Dra I	TTTAAA
Xba I	TCTAGA
*Hae*Ⅲ	GGCC
Alu I	AGCT
Sal I	GTCGAC
*Bam*H I	GGATCC
Pst I	CTGCAG
EcoR I	GAATTC
Xho I	CTCGAG
Msp I	CCGG
Apa I	GGGCCC

On the other hand, there were some differences in digestive properties among the 6 kinds of the restrictive enzymes as shown in Fig. 2. These findings demonstrate that the digested DNAs contain the respective recognition sequences and vice versa. The DNAs from *P. tenera* (Minomi), *P. tenera* (Matsukawa), *P. yezoensis* (Saga10) and *P. yezoensis* (Green) except *P. yezoensis* f. *narawaensis* and *P. seriata* (Shimabara) were digestible with *Bam*H I and *Pst* I. Those from *P. tenera* such as *P. tenera* (Minomi) and *P. tenera* (Matsukawa) were not digested by *Eco*R I, but the other DNAs could be digested. For *Xho* I, DNA from a wild species, *P. seriata* (Shimabara) was specifically digested but those from other cultivated species, *P. tenera* and *P. yezoensis* were not. *Msp* I specifically digested DNAs from all the species but *P. yezoensis* f. *narawaensis*, whereas *Apa* I digested only DNAs from *P. tenera* (Minomi) and *P. yezoensis* (Green), but not those of other species.

In conclusion, *Porphyra* can be classified biochemically from the results of DNA digestion with 6 kinds of restriction enzymes ; *Bam*H I, *Pst* I, *EcoR* I, *Xho* I, *Msp* I, and *Apa* I.

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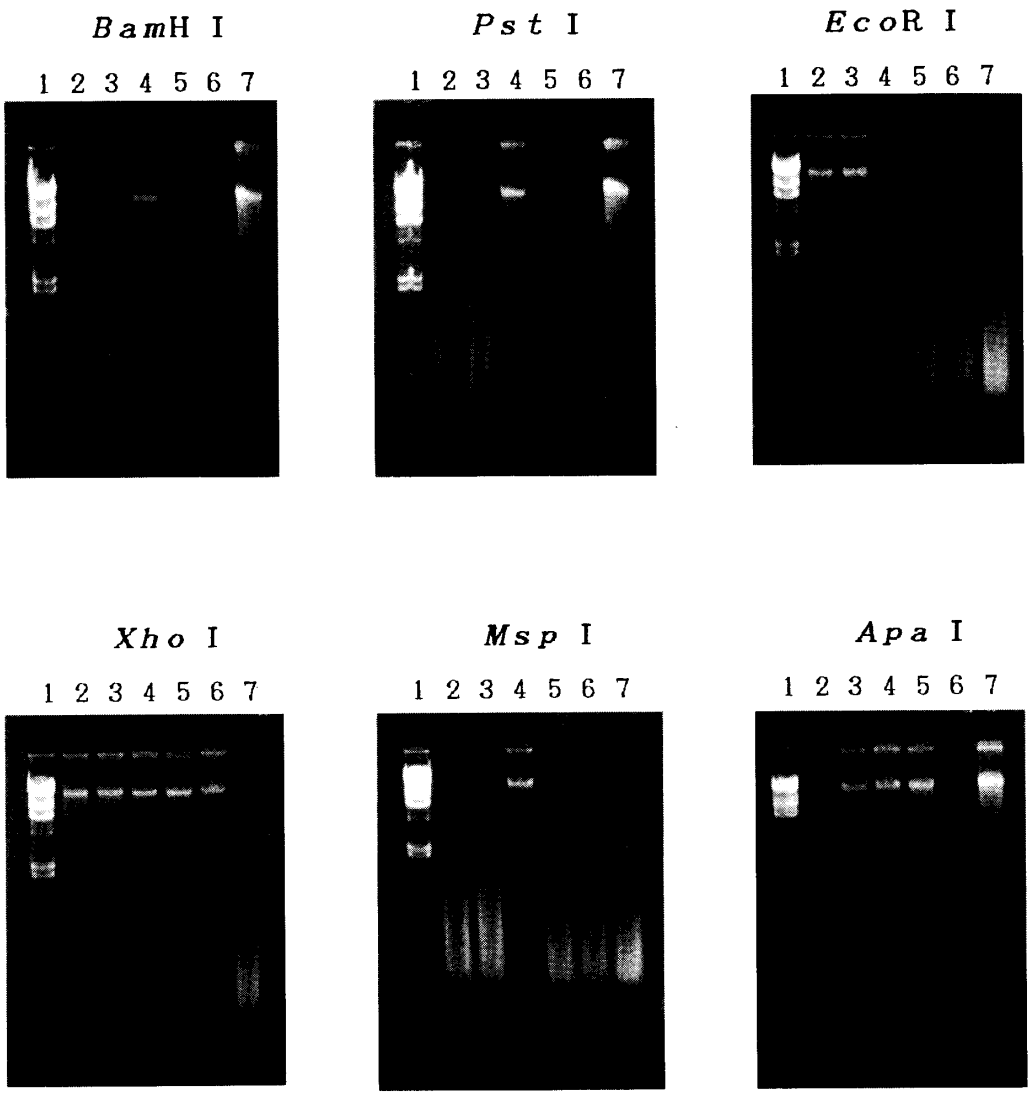

Fig. 2 Electrophoretic patterns of DNAs from different species of *Porphyra* conchocelis, after digestion with several restrictive enzymes.
Lane 1 ; *Hind*Ⅲ-digested λ DNA, Lane 2 ; *P. tenera* (Minomi),
Lane 3 ; *P. tenera* (Matsukawa), Lane 4 ; *P. yezoensis* f. *narawaensis*,
Lane 5 ; *P. yezoensis* (Saga10), Lane6; *P. yezoensis* (Green),
Lane 7 ; *P. seriata* (Shimabara)

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