

## Use of minisatellite probes for DNA fingerprinting of cultivated laver

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Two probes known to allow DNA fingerprinting in mammals, wild type M13 phage DNA and Jeffreys' human minisatellite sequence (myo probe), were examined for their ability to reveal DNA variation in two laver cultivars. Hybridization of the laver DNA with both probes revealed distinct hybridization bands of high molecular weight restriction fragments. The hybridization patterns of restriction fragments were slightly different between the two cultivars examined. These results suggest that minisatellite sequences similar to M13 DNA and Jeffreys' myo probe are involved in the laver genome, and genetic variation among the cultivated lavers can be detected in the DNA fingerprints using these minisatellite sequences.

### 1 Introduction

The red algae *Porphyra yezoensis* and *P. tenera*, usually called "laver", are important marine products in Japan. Until now, many cultivars or cultivated species of laver have been isolated, stocked and widely cultured in coastal farms. Laver cultivars were, in many cases, discriminated from each other on the bases of morphological, color and growth characteristics. However, these characteristics sometimes change under differing growth conditions, which has resulted in some ambiguity in the discrimination of laver cultivars. On the other hand, biochemical means have been recently introduced for the discrimination of laver cultivars or the identification of strains and species of *Porphyra*<sup>1~3)</sup>.

DNA fingerprinting techniques based on restriction fragment length polymorphism (RFLP) have been recently applied to identify the species, strains, cultivars and even individuals of various organisms<sup>6,7)</sup>. The minisatellite DNA is a well known probe used in Southern blotting experiments of RFLP analysis.

Minisatellite DNA is composed of tandem arrays of short core sequences and is scattered throughout the chromosomal DNA<sup>8,9)</sup>. Variation in the number of core sequences provides numerous alleles, and therefore, reveals extensive genetic polymorphism. M13 phage genome DNA (M13 DNA)<sup>5,10,11)</sup> and the 33 base pair(bp) repeat sequence in human myoglobin gene (Jeffreys' myo probe)<sup>4,8,12)</sup> are well known minisatellite DNA which allow fin-

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gerprinting in various animals and plants.

We previously reported that fingerprinting with synthetic microsatellite probes was useful for the discrimination of laver cultivars<sup>13)</sup>. In the present study, we examine whether minisatellite sequences derived from M13 DNA and Jeffreys' myo probe are useful for detecting genetic variation in laver cultivars.

## 2 Materials and Methods

### 2.1 Algae

*Porphyra yezoensis* cultivar Noma and *P. tenera* cultivar Oba-asakusa (Oba) were used for RFLP analysis with minisatellite DNA probes. These algae were produced by the Saga Prefectural Ariake Research and Development Center and Fukuoka Fisheries and Marine Technology Research Center, Ariake Institute, and were cultured at a sea farm in Ariake Bay, Japan.

### 2.2 DNA extraction and hybridization

Protoplasts were prepared from thalli samples (2 to 3 g of wet weight, consisting of about 400 to 700 thalli shorter than 5 cm in length) as described previously<sup>14)</sup>. They were lysed in lysis buffer (0.4M sucrose, 0.5% Triton X-100, 80 mM KCl, 50mM Tris-HCl, pH 7.5). The lysate was filtered through a 40  $\mu$ m-mesh nylon gauze and centrifuged at 8,000g for 20min. DNA was extracted from the resultant nuclear-rich pellet in extraction buffer (0.1M NaCl, 5mM EDTA, 20mM Tris-HCl, pH7.5) by phenol/chloroform methods as described previously<sup>13)</sup>. DNA was precipitated in 2.5 volumes of cold ethanol, washed with 80% ethanol, vacuum-dried and dissolved in 10mM Tris-HCl-1mM EDTA (pH7.5).

Approximately 12  $\mu$ g of laver DNA was digested with 5 to 10-fold excess units of restriction enzymes in a total volume of 150  $\mu$ l. The digested DNA was extracted once with phenol/chloroform (1:1), once with chloroform, precipitated with ethanol and dissolved in 15  $\mu$ l of 10mM Tris-HCl-1mM EDTA

(pH7.5) after being dried. DNA solution was loaded onto 1.2% agarose gels and electrophoresed at 1 V/cm for 32 to 36hours with recirculating running buffer using a peristaltic pump. Gels were stained with ethidium bromide and photographed under UV light, then deprimated in 0.25M HCl for 15min, denatured in 0.5M NaOH/1.5M NaCl for 20min and neutralized in 0.5M Tris-HCl (pH7.5)/1.5M NaCl for 20min. DNA was transferred from agarose gel to nylon membrane by Southern blotting in 20 $\times$ SSC. The membrane was hybridized with minisatellite probe which was labeled with fluorescein-11-dUTP using the Amersham ECL-labeling system (Amersham Co., UK). After hybridization, the membrane was washed in 6 M urea-0.4% sarcosyl-0.5% SSC for 10min at room temperature, then twice in 2  $\times$  SSC for 5min and followed by autoradiographies. The procedures of DNA labeling and chemiluminescent detection of labeled DNA followed the ECL-labeling system (Amersham) manufacturer's protocol.

### 2.3 Probes

Two probes, M13 DNA and Jeffreys' myo probe, were used in the present study. The M13 DNA was obtained as M13 phage mp18 DNA from Takara Shuzo (Kyoto, Japan). The myo probe was isolated from plasmid pUC-myo, which was kindly provided by Dr. Kominami, Department of Biochemistry, Niigata Univ., School of Medicine, Niigata, containing approximately 15 tandem copies of Jeffreys' 33-bp repeat sequence<sup>8)</sup> of the human myoglobin gene at the Sma I site of plasmid pUC 19.

## 3 Results and discussion

Total DNA was extracted from cultivars Noma and Oba, each of which was believed to belong to the species of *Porphyra yezoensis* and *P. tenera*, respectively. DNA was digested with restriction enzymes Hae III, Mva I or Rsa I, transferred to membranes and hybridized with M13 DNA probe or

Jeffreys' myo probe. As described in the materials and methods section, the laver DNA was extracted from protoplasts which were prepared from frozen laver samples consisting of 400 to 700 thalli. Therefore, hybridization patterns of the DNA revealed the characteristics of cultivars or species rather than individuals thalli. Our unpublished preliminary experiments showed that the clearest resolutions of restriction fragments were obtained using the restriction enzymes Hae III, Mva I and Rsa I when 15 restriction enzymes were attempted (data not shown). In addition, preliminary experiments revealed that the fingerprints obtained with the above probes were very similar between Noma and Saga-5. Saga-5 is one of typical cultivar used in laver production,

and is believed to be a cultivar of *Porphyra yezoensis* similar to cultivar Noma (data not shown). Therefore, only Noma was used in the comparison of DNA fingerprints with cultivar Oba which is believed to belong to *Porphyra tenera*, in the present study.

Fig. 1 shows DNA fingerprints of Noma and Oba generated with M13 DNA as a probe. Although clusters of poorly resolved DNA bands are seen at the low molecular weight regions, distinct DNA bands of high molecular weight restriction fragments can be seen in the patterns of both Noma and Oba DNA. These high molecular weight bands presumably represent minisatellite loci in laver genomes. The labeled DNA bands were rare in the high mo-

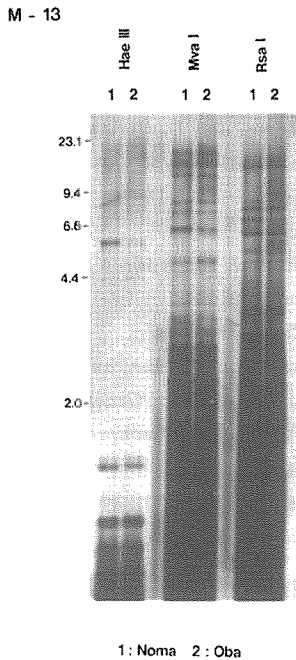


Fig. 1 DNA fingerprints of laver DNA generated with M13 DNA probe. DNA was extracted from cultivars Noma and Oba, digested with Hae III, Mva I and Rsa I, electrophoresed and hybridized with wild type M13 DNA as probe. 1, Noma; 2, Oba. Size markers on the left are given in kb.

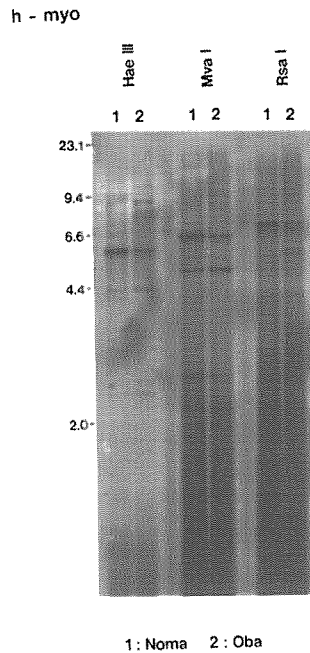


Fig. 2 DNA fingerprints of laver DNA generated with Jeffreys' myo probe. DNA of Noma (1) and Oba (2) were analyzed as described in Fig. 1 using Jeffreys' myo probe instead of M13 DNA. Size markers on the left are given in kb.

lecular weight region of the hybridization pattern, which was probably brought about by the considerably smaller genome size in laver than those in higher animals<sup>15)</sup>. The distinct and thick 5.8kb band was detected in the Hae III digestion of Noma DNA but only faint 5.8kb band in Oba DNA, whereas 9.8kb Mva I and 9.5 and 5.6kb Rsa I fragments were seen in Oba DNA but not in Noma DNA. These differences in hybridization pattern may suggest that genetic variations occur between Noma and Oba, which can be detected by the M13 DNA probe. Fig. 2 shows hybridization pattern of Noma and Oba DNA, probed with myo probe. The fingerprints of Rsa I and Hae III digested DNA were slightly different between Noma and Oba; the 5.8kb Rsa I and 7.8kb Hae III fragment were found only in Oba DNA, suggesting that polymorphism of laver DNA can be also detected with myo probe.

Because of the difficulty of artificial fertilization in laver, we could not directly ascertain the inheritance of the polymorphic DNA fragment appeared in Fig. 1 and Fig. 2. However, we previously demonstrated that DNA fingerprints obtained with microsatellite probes were identical among several laver samples which were identical in cultivar but cultured and harvested in different years<sup>13)</sup>. Therefore, we suggest that the DNA fingerprints shown above are genetically significant and laver DNA polymorphism can be detected with minisatellite sequences of M13 DNA and myo probe. It remains to be solved whether these minisatellite DNA are practical for the discrimination of many laver cultivars in fingerprint analyses. For this purpose, it will be necessary to apply DNA fingerprinting to many laver cultivars using minisatellite probes.

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#### ミニサテライトプローブを用いたアマノリDNAのフィンガープリント

水上 讓・岡内正典・小林正裕・鬼頭 鈞

2種類のミニサテライトプローブ、M-13フェージDNA及びジェフレイのミオプローブは、動物等のDNAフィンガープリントに利用されている。これらのプローブがアマノリDNAの多型検出にも有効かどうか制限酵素切断片鎖長多型解析によるフィンガープリントによって検討した。その結果、いずれのプローブを用いた場合でも、濃いハイブリダイゼーションバンドと2品種間で異なったハイブリダイゼーションパターンが検出され、アマノリゲノム中にミニサテライトと類似の塩基配列が散在していること、また、品種間多型がこれらのプローブによって検出できることが示唆された。