

Principal Research Results

Identification of Fouling Species in Barnacle Larvae by DNA Analysis

Background

A large quantity of fouling sessile organisms, especially barnacles invade cooling water systems and cause serious problems such as lower water intake, blockade and corrosion in the cooling systems and disposal of waste at several electric power stations in Japan. The fouling barnacle species are limited but it is difficult to identify the species of larvae by their morphological features. If larvae of the fouling species are detected before their settlement, it is possible to forecast the settlement period and to take measures effectively (Fig.1).

Objectives

The aim of the present study is to develop an easy and precise method for identification of fouling species in barnacle larvae by DNA analysis.

Principal Results

1. Species identification of Japanese common barnacles by DNA sequences

Fifteen common barnacle species inhabiting the Japanese coast were collected. Total DNA was extracted from each adult barnacle, and PCR (polymerase chain reaction) was carried out using the specific primer pair for 12S rRNA gene on the mitochondrial genome. DNA sequences of analysis region in 12S rRNA gene were compared, and inter-species differences were found to be remarkable in comparison with intra-species variations (polymorphism). These results show that the barnacle species can be identified by the sequences of 12S rRNA gene (Fig.2).

2. Development of DNA analysis for individual larva

We developed a simple direct PCR method without DNA extraction for individual larva. Individual larva was immersed in 99.5% ethanol, dried, and applied to direct PCR. This method worked very well, and DNA sequence data were obtained in almost 100% of larval specimens.

3. PCR-RFLP* 1 for detection of *Megabalanus rosa*

From the sequence data of 15 species, a specific restriction site for the enzyme, *Spe I*, was found only in the sequence of *Megabalanus rosa*, the principal fouling species in Japan (Fig.2). In fact, the results of PCR-RFLP showed that two bands of DNA fragments cut by *Spe I* were clearly detected only in the specimens of *Megabalanus rosa* (Fig.3). It is possible to detect the larvae of fouling species within one day by this method.

4. Identification of the larvae of *Megabalanus rosa* in mixed plankton samples from natural sea

The PCR-RFLP method developed in the present study was applied to mixed plankton samples collected from natural sea, and was revealed to be valid for identification of *Megabalanus rosa* larvae in the field (Fig.4).

Future Developments

On the basis of the present study, the quantitative and simple detection method for larvae of the fouling barnacles in mixed plankton samples will be developed in order to establish more practical technology.

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Reference

K. Matsumura, et.al., 2006, "Identification of fouling species in barnacle larvae by DNA analysis", CRIEPI Report V05007, (in Japanese)

* 1 : PCR-Restriction Fragment Length Polymorphism

2. Environment - Coexistence with the environment



Fig.1 Necessity to develop precise method for species identification of barnacle larvae

It is difficult to identify the species of barnacle larvae by their morphological features. If larval species of fouling barnacle are easily detected before settlement, the measures will be taken effectively.

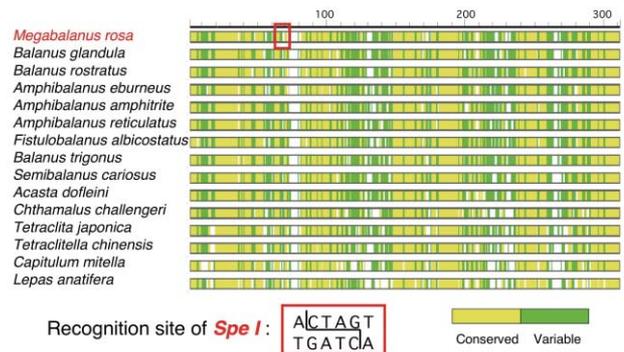


Fig.2 Inter-species variation of 12S rRNA gene in 15 barnacle species and restriction site of *Spe I*

DNA fragments of 12S rRNA gene were amplified by PCR, and the sequences were determined in 15 common barnacle species. The *Spe I* recognition sequence was found only in *Megabalanus rosa*.

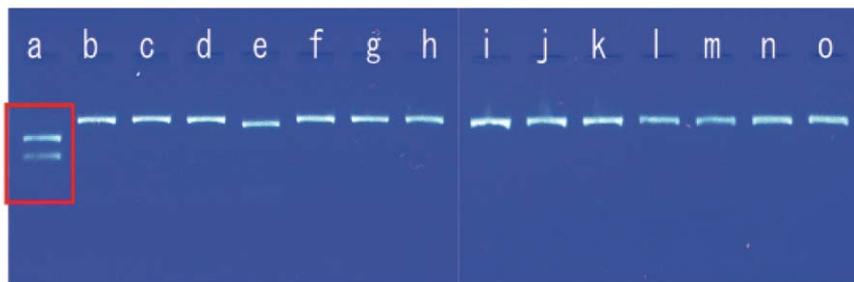


Fig.3 PCR-RFLP using *Spe I* for 15 common barnacle species

Each PCR product of 15 barnacle species was treated with the restriction enzyme, *Spe I*, and was applied to agarose gel electrophoresis to visualize the DNA fragments. The results show that two bands of fragments were clearly detected only in *Megabalanus rosa* (a).

a: *Megabalanus rosa* **b:** *Balanus glandula* **c:** *Balanus rostratus* **d:** *Amphibalanus eburneus*
e: *Amphibalanus amphitrite* **f:** *Amphibalanus reticulatus* **g:** *Fistulobalanus albicostatus*
h: *Balanus trigonus* **i:** *Semibalanus cariosus* **j:** *Acasta dofleini* **k:** *Chthamalus challengeri*
l: *Tetraclita japonica* **m:** *Tetraclitella chinensis* **n:** *Capitulum mitella* **o:** *Lepas anatifera*

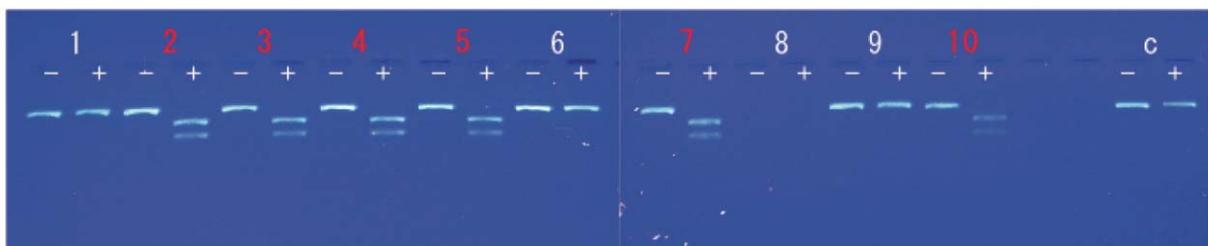


Fig.4 PCR-RFLP using *Spe I* of larval samples collected from natural sea area

1~10: individual larval specimen isolated from collected plankton samples
 c: control (*Balanus rostratus* nauplius cultured in the laboratory)
 -: untreated PCR product, +: *Spe I*-treated PCR product
 Larvae of No. 2, 3, 4, 5, 7 and 10 were estimated to be *Megabalanus rosa*.