Review

Genetic and Breeding Science of Abalone and their Application to the Aquaculture Techniques

Motoyuki HARA*1 and Masashi SEKINO*2

(Accepted April 13, 2001)

Abstract: To promote genetic researches and breeding science of Pacific abalone, we are beginning to develop the isolation and characterization the hyper-variable markers such as microsatellite DNA. Microsatellite DNA markers are expected to provide an invaluable tool for this purpose because of the power and ability of microsatellite DNA markers in regard to resolution for genetic relatedness among individuals or populations. Here, we describe the applications to the aquaculture techniques of abalone using microsatellite DNA markers. Comparing the genetic variability of microsatellite DNA with that of allozyme in natural and artificial populations, the variability observed in microsatellite DNA analysis was evidently higher than that observed in allozyme analysis. The fact was suggesting that the microsatellites DNA should prove useful as sensitive genetic markers to estimate genetic variability. As the application to the discrimination of individuals, the estimation of the traits of pedigree in mixed rearing populations was initiated to establish using five microsatellite DNA markers in Pacific abalone. The group that was strong to withering syndrome was found from survival groups against the disease. The feasibility of pedigree analysis using microsatellite DNA markers could be ascertained. It is considered that the development of abundant number of microsatellite DNA markers in abalone promote the genetics and breeding science and become one of the most useful approaches to develop the aquaculture techniques.

Key words: Genetic variability; Pacific abalone; Microsatellite DNA; Pedigree analysis

Introduction

Abalone is one of the most expectant species that their products are increased using the aquaculture techniques. One of the reasons is more than twenty million seeds of them have been produced by artificial fertilization and cultured before twenty years, and another reason is high price in market. However, the problems of the typical low growth rate and the mass death accompanying with withering syndrome in the procedure of seeds mass-production have been prevented to develop the abalone culture remarkably. One of the approaches to resolve the problems is the application of the breeding techniques of selection and crossing etc. The selection response for growth rate in Pacific abalone were reported by Hara1) and Kawahara et al.2). On the other hand, hyper-variable genetic markers, such as microsatellite DNA markers have been developed rapidly accompanying with the advance of molecular biology, and they have been begun to the apply to the effective breeding techniques. And further, Microsatellite DNA markers are also expected to provide an invaluable tool for various purposes in fish and shellfish research fields. Because the power and ability of microsatellite DNA markers could be resolved precisely the genetic relatedness among individuals or populations.

Here, we introduce the method of the efficient isolation of microsatellite DNA markers from

*1 National Research Institute of Aquaculture, Nansei, Mie, 516-0193, Japan.
*2 Tohoku National Fisheries Research Institute, Shiogama, Miyagi, 985-0001, Japan.
Pacific abalone, and describe the availability for the evaluation of the genetic diversity of natural and artificial populations using microsatellite DNA markers. And further, we estimate the feasibility of the pedigree tracing using hypervariable microsatellite DNA markers, and establish the method of the evaluation of genetic characters in mixed rearing individuals.

**Isolation and characterization of microsatellite DNA loci in Pacific abalone Haliotis discus discus**

An efficient method to clone chicken microsatellites has been reported by Takahashi et al.\(^3\), and Sekino et al.\(^4\) succeeded in its application to teleost fishes with modifications. We applied the isolation and characterization of microsatellite DNA loci to Pacific abalone. In brief, genomic DNA was fragmented by sonication, and sonicated fragments were blunted by mung bean nuclease. The fragments ranging about 400bp were ligated into Srf site of PCR-Script Amp SK (+) vector, and the recombinant plasmid vector was transformed into XL2-Blue MRF\(^\prime\) ultracompetent cells Single-stranded DNA was prepared, and selective second-strand DNA synthesis was employed using (CA)\(_{12}\) oligonucleotide and cloned pfu DNA polymerase. The resultant double-strand DNA was transformed into XL2-Blue MRF\(^\prime\) cells again and these transformants were referred to a (CA)\(_n\)-enriched library. Dot-blot hybridization analysis using DIG labeled (CA)\(_{10}\) oligonucleotide probe and DIG nucleic acid detection kit revealed that 41 of randomly chosen 48 clones from the library gave positive signals (approximately 85\%). Considering the efficiency of recovery for (CA)\(_n\) repeats in a non-enriched library was approximately 0.2% (data not shown), and assuming the proportion of duplicated clones is not significant, the method used in this study achieved an overall 400-fold enrichment. We chose 39 clones from the library without complicated colony hybridization screening steps, and plasmid DNAs were purified. The DNA sequences were determined using Thermo Sequenase cycle sequencing kit in combination with KS and T3 primers and subjected to an automated DNA sequencer. Of the 39 clones, independent 41 (CA)\(_n\)-microsatellite repeats were detected. The length of continuous CA-repeat units varied between 5 and 41, with a mean value of 12.1. We developed 28 primer sets using a Primer Premier software package (Premier Biosoft International, Palo Alto, CA, USA). Primer sets were tested for amplification effectiveness in Pacific abalone. Genomic DNA was extracted from shell muscle of each individual (Asahida et al.,\(^1\), 1996). PCR amplification was performed in a 20\(\mu\)l reaction volume, which included 20 pmols of each primer (one of a pair primer was 5’ end-labelled with Cy5), 100\(\mu\)M each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl\(_2\), 0.001% gelatin, 0.5 units of Taq, and approximately 200 ng of template DNA using a thermal cycler. PCR amplification cycles were as follows: 12 min at 94°C, 40 cycles of 30 sec at 94°C, 45 sec at a primer-specific temperature, and 45 sec at 72°C, and final elongation for 7 min at 72°C. An estimate of microsatellite polymorphism in Pacific abalone was obtained by screening 20-36 individuals using the DNA sequencer in combination with an Allelelinks software package (Amersham Pharmacia Biotech). We finally chose five primer sets (Table 1) because the remaining primer sets have been nonpolymorphic or produced unexpected PCR products (Sekino and Hara\(^6\), in press).

**Comparison of genetic variability between microsatellite DNA and allozyme makers in natural stock for release and selected populations**

To investigate the availability of microsatellite DNA makers, we analyzed the genetic variabilities of natural, released and cultured populations, comparing to allozyme data (Table 2). On the result analyzed by microsatellite DNA makers, the numbers of alleles per loci on the artificial populations were different with three populations (natural, released and cultured populations). And the values of the natural, released and cultured populations were 11.3, 9.9 and 5.7 respectively. The expected heterozygosity was similar to the number of alleles, the expected heterozygosities of the natural, released and cultured populations were 0.723, 0.668 and 0.543 respectively. The expected heterozygosities of the released and cultured populations...
were clearly decreased in compare with that of the natural population. The reduction of number of alleles and the expected heterozygosity were caused to loss rear alleles in reproductions attributed to a limited number of founders of the hatchery stock. These results were consistent with the estimation that the variability of artificial populations was lower than that of natural population. On the other hand, these values of allozyme analysis reported by Kijima et al.\(^7\) were slightly different between natural and released populations, showing in Table 2. The variability observed in microsatellite DNA analysis was evidently higher than that observed in allozyme analysis, and suggesting that these microsatellites should prove useful as sensitive genetic markers to estimate genetic variability.

**Evaluation of survival trait for withering syndrome using pedigree analysis based on microsatellite DNAmakers**

We have been initiated to assess the feasibility of establishing pedigree in mixed rearing populations. Withering syndrome is a fatal disease in the mass production of abalone artificial seeds. The A group (survivor group) was made from the seeds that were survived when withering syndrome was caused. The B group (natural group) was made from natural individuals that were not selected. Further, the C group (hybrid group) was crossed the A group with the B group. The three groups have been mixed after soon fertilization and reared. The many experimental seeds died with withering syndrome from May to August. The ratio and number of individuals on the three groups was shown in Table 3. The three groups could be identified using five microsatellite DNA makers. The ratio of the A group sired by the survivor parents was increased from 25% to 53.3% largely. However, the ratio of the B group produced by the natural parents was greatly decreased from 49.7% to 20.6%. And also, the ratio of the C group was decreased from 33.3% to 26.2%.

The averages of shell lengths on the three group (A, B and C) were shown in Table 4. The average of shell length of the A group was smaller than that of the other two groups (B and C) in May. However, the averages of shell length on all groups became almost same in August. It was ascertained that the groups in the mixed rearing could be discriminated by means of hyper-variable microsatellite DNA

---

### Table 1. Five microsatellite loci isolated from the Pacific abalone H. discus discus. Ho is observed and He is expected heterozygosity

<table>
<thead>
<tr>
<th>Locus</th>
<th>Core repeat sequence (5'-3')</th>
<th>Primer sequence (5'-3')</th>
<th>Anneal (°C)</th>
<th>Expected Size (bp)(^1)</th>
<th>GenBank accession no.(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hdd6C</td>
<td>(GACT)(_2)(CTCA)(_7)(CA)(_2) CT(CA)(_9)</td>
<td>F-TTTAAGACCAACTTGTAGACATCC R-ACGGCCGCTTGGTGTTTG</td>
<td>57</td>
<td>123</td>
<td>AB025367</td>
</tr>
<tr>
<td>Hdd108C</td>
<td>(CA)(_{30})</td>
<td>F-AGCTGTATACCCAGATCCG R-GCCATTGTAGAGGATCGAGGAGGA</td>
<td>50</td>
<td>174</td>
<td>AB025384</td>
</tr>
<tr>
<td>Hdd114B</td>
<td>(CA)(<em>2)(CT(CA))(</em>{12})(CGCA)(_{11}) (CA)(_6)</td>
<td>F-ACCTGACAGCAGGAACTTCT R-GTGGCAGCCGCGTCTGGTGA</td>
<td>58</td>
<td>234</td>
<td>AB025387</td>
</tr>
<tr>
<td>Hdd115B</td>
<td>(CA)(_8)(CG)(_4)</td>
<td>F-CTAAATTTAAACAGCCGGTATGA R-TTATGTTAAAGATCGGAGGTTCAG</td>
<td>50</td>
<td>245</td>
<td>AB025388</td>
</tr>
<tr>
<td>Hdd229</td>
<td>(TCA)(_8)(AT)(_7)(GA)(<em>9) (CA)(</em>{16})(CTCA)(_8)(GA)(_5)(CA)(_3)</td>
<td>F-TAGCTAAACGCGAGCAGA A-ACCGCCCGCGCTAATATACAC</td>
<td>59</td>
<td>210</td>
<td>AB047107</td>
</tr>
</tbody>
</table>

\(^{1}\)Size is indicated as number of the base pairs of PCR products.

\(^{2}\)The nucleotide sequence data will appear in the DDBJ/EMBL/GenBank nucleotide databases with the accession numbers.
Table 3. Proportion(%) and individual number of three group in May and August

<table>
<thead>
<tr>
<th>Group</th>
<th>May (% (No.))</th>
<th>August (% (No.))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (survior)</td>
<td>25.0 (18)</td>
<td>53.7 (57)</td>
</tr>
<tr>
<td>B (natural)</td>
<td>41.7 (30)</td>
<td>20.6 (22)</td>
</tr>
<tr>
<td>C (hybride)</td>
<td>33.3 (24)</td>
<td>26.2 (29)</td>
</tr>
</tbody>
</table>

*1 Shell length was calculated on ratio of small medium and large size.

Table 4. Average of shell length (mm) of the three group in May and August

<table>
<thead>
<tr>
<th>Group</th>
<th>May *1</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (survior)</td>
<td>7.6</td>
<td>16.5±4.78</td>
</tr>
<tr>
<td>B (natural)</td>
<td>10.4</td>
<td>16.3±3.91</td>
</tr>
<tr>
<td>C (hybride)</td>
<td>10.5</td>
<td>15.1±4.01</td>
</tr>
</tbody>
</table>

marker, the evaluation of the genetic characters such as resistance for diseases was practiced effectively. And further, it is suggesting the possibility that the A group is resisted to withering syndrome. Nevertheless, analysis of parentage could not been accomplished yet using only five microsatellite DNA makers.

It was ascertain that microsatellite DNA markers was hyper-variable genetic markers with great discriminating power for genetic researches in Pacific abalone. Of cause, the five microsatellite DNA marker's loci were not enough for population research and pedigree analysis. It is considered that the development of abundant number of microsatellite DNA loci in abalone promote the genetics and breeding science and become one of the most useful approaches to develop the aquaculture techniques.

Acknowledgements

We express gratitude to the Japan Aquaculture Society for giving the opportunity to publish this manuscript in the special issue of SUISAN-ZOSHOKU. We thank to the staff in Mie Prefectural Fish Farming Center, and Fisheries Co-operative Association of Misakicho, Kesencho, Hirotacho, Kamaishitoubu and Tarocho for the offer of the abalone samples.

References


アワビの遺伝育種研究と養殖技術への応用

原 素之・関野正志

マイクロサテライト DNA（MS）マーカーは変異性の高さや実験再現性の良さから、種々の遺伝的調査や効率的育種を進めるための有効なツールとして期待されている。しかし、MSマーカーは種特異的な場合が多く、分析対象種ごとに煩雑なマーカーの開発が必要であり、その普及を阻んでいる。我々は、効率的なMSマーカー単離法を検討しながらアワビ類のMSマーカーを開発し、これを用いて、エゾアワビの遺伝的多様性評価やクロアワビの家系判別による形質評価への応用を試みた。その結果、アロザイムと比べ明らかに変異検出感度が高く多様性評価に有効であることが確認された。また、受精時から混合飼育した種苗について家系判別を行い、筋萎縮症発症時の生残グループの耐性を遺伝的に明らかにした。以上のことから、MSマーカーはアワビの遺伝育種研究においても有用であることを示した。