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Cross-species Amplification of Scallop Microsatellites in the Family Pectinidae

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Abstract: In the present study, 103 microsatellite markers were selected for cross-species amplification in four marine economic scallop species (*Argopecten irradians, Chlamys farreri, Chlamys nobilis,* and *Mizuhopecten yessoensis*) belonging to the family Pectinidae. As a result, in the 72 markers originated from *C. farreri*, 12 showed amplification products in *C. nobilis*, 12 in *A. irradians* and 11 in *M. yessoensis*. In the 12 markers developed from *M. yessoensis*, four markers could be amplified successfully in *A. irradians*, three in *C. farreri*, and one in *C. nobilis*. In the nine markers isolated from *C. nobilis*, two markers could be cross-amplified in *A. irradians* and one in *M. yessoensis*, but no marker was likely to be useful in *C. farreri*. In the nine markers of *A. irradians,* one marker was potentially workable for *C. farreri,* one in *M. yessoensis*, but none in *C. nobilis*. When the microsatellites were cross-species amplified, most of the PCR products showed low yield and ambiguous bands, while the numbers of alleles also decreased. Finally, three markers (CFMS016, CFCD131 and CFE04) were revealed to be successfully transferred among the four species providing candidate markers for ecological study of scallops, while most of the other markers were unique for one species with poor cross-species amplification, which might be useful for species identification.

Keywords: Cross-amplification, Genetic Marker, Scallop, Species Identification, Simple Sequence Repeats

1. Introduction

Scallops *Argopecten irradians*, *Chlamys farreri*, *Chlamys nobilis* and *Mizuhopecten yessoensis* are species belonging to the different genera of family Pectinidae, which are all important economic shellfish in China. Their living conditions are heterogenous, which determines their distribution areas. It is important for the ecologists to study the genetic diversity of the four species by molecular markers in order to protect the scallop resources.

Microsatellite DNA markers, with high polymorphism and codominance features, have been widely used in genetic analysis [1-5]. However, there are also many disadvantages caused by the costs involved in isolating, cloning, sequencing and characterizing microsatellite loci from the target species being examined for the first time. The discovery of microsatellite markers in related species provides an alternative approach to developing microsatellites in a simple and direct way. Transferabilities of microsatellite markers in related species have been studies in many shellfish, such as in *Crassostrea gigas* [6] and *Haliotis corrugata* [7].

As for scallop, a large number of microsatellites have been developed for the four species [8-11]. In this paper, 103 microsatellite DNA marker's transferability was further evaluated in the four scallop species in order to develop potential markers for related species, especially to find markers that can be amplified in all four species to facilitate the ecology investigation and even to use them to evaluate the phylogenetic relationships of scallops.

2. Materials and Methods

2.1. Sampling and DNA Extraction

For transferability analysis, six individuals of each scallop species, *A. irradians*, *C. farreri*, *C. nobilis* and *M. yessoensis* were selected, which were popularly cultured in the seacoast of China. Among them, *C. farreri* and *C. nobili* were collected from the natural habitats in Changdao City (Shandong Province) and Dongshan City (Fujian Province) of China,

respectively. The two introduced species, *M. yessoensis* and *A. irradians*, were collected from the Yantai City (Shandong Province). The live individuals were shipped to the laboratory, and the adductor muscles were taken and stored in the –80°C refrigerator. Genomic DNA was extracted from adductor muscle using traditional phenol-chloroform method [12].

2.2. Microsatellite Markers

Among the 103 markers that were tested in cross-specific PCR amplifications, 72 markers came from *C. farreri* [8, 13, 14], 12 from *M. yessoensis* [9], nine from *C. nobilis* [10] and nine developed from *A. irradiant* [15]. The primer and microsatellites information could be found in the publications above.

2.3. Cross-species Amplifications

In order to ensure the amplification accuracy of these species-specific markers, the annealing temperature for transferability was adopted with a wide range: 40°C to 62°C. PCR amplifications were set up in a 20 μL volume composed of 100 ng of genomic DNA (3 individuals mixed), 0.2 μM of each primer, 200 μM of each dNTP, 1 U *Taq* polymerase (Takara) and 1 x universal PCR buffer. Thermal cycling was performed in a Biometra T-gradient Thermal Cycler System. The PCR program was used as following: 5 min at 95°C for initial denaturation; followed by 35 cycles of 30 sec at 95°C, 30 sec at gradient temperature, 45 sec at 72°C and a final extension at 72°C for 5 min. PCR products were separated by electrophoresis on 10% non-denaturing polyacrylamide gel in 1 x TBE buffer, and the gels were visualized by ethidium bromide staining. The amplification results were recorded.

The PCR product bands were classified into four types: (1) clear amplified (marked with optimized annealing temperature); (2) unorderly band; (3) weak band with few product; (4) No product.

2.4. Polymorphism Evaluation of the Four Species Transferable Markers

After recording, only the markers which could be amplified in all four species were selected to evaluate their allele numbers in six individuals of each species. The annealing temperature was re-optimized for them. The PCR amplification system was the same as above and the products were detected by 10% non-denaturing polyacrylamide gel as well and the results were written down by hand.

3. Results

3.1. Cross Species Amplification

In the 72 markers originated from *C. farreri*, PCRs of 11 markers yeilded clear amplification products in *M. yessoensis*, 12 in *C. nobilis* and 12 in *A. irradians*. In the 12 markers developed from *M. yessoensis*, three markers could be well amplified in *C. farreri*, one in *C. nobilis* and four in *A. irradians*. In the nine markers isolated from *C. nobilis*, no marker was likely to be useful in *C. farreri*, one in *M. yessoensis*, two in *A. irradians*, while among the nine markers isolated from *A. irradians,* one marker was potentially workable in *C. farreri,* one in *M. yessoensis*, but none in *C. nobilis*. Markers which were successfully amplified were given new annealing temperatures (Table 1). However, when amplified in related species, many of the PCR products showed low yield and ambiguous bands (Table 1).

Table 1. *The cross-species amplification results in four scallop species.*

Loci	Amplification results in four species				
	C. farreri	*M. yessoensis*	*C. nobilis*	*A. irradians*	Transferbility in four species
C. farreri loci					
CFAD239	+	-	W	49.0°C	
CFAD196	+	M	53.8°C	M	
CFAD080	+	W	W	M	
CFAD139	+	-	-	-	
CFAD019	+	-	-	-	
AD195	+	-	-	-	
13RB01*	+	45.5°C	45.5°C	45.5°C	√
15RB04*	+	45.5°C	M	45.5°C	
R5H4*	+	M	45.5°C	61.0°C	
19RH06*	+	45.5°C	W	M	
13RB04*	+	M	M	M	
22FG07*	+	W	W	61.0°C	
20RB03*	+	-	-	-	
CFAD099	+	-	-	-	
CFCD126	+	W	M	53.8°C	
CFE26	+	-	-	-	
CFCD134	+	W	W	M	
CFE23	+	-	-	-	
CFE20	+	56.7°C	-	M	
CFE19	+	M	-	S	
CFE15	+	M	-	-	
CFE18	+	W	50.5°C	M	
CFE12	+	50.0°C	W	W	
CFE11	+	W	-	S	

Loci	Amplification results in four species				
	C. farreri	*M. yessoensis*	*C. nobilis*	*A. irradians*	Transferbility in four species
CFE07	+	58.6°C	58.6°C	W	
CFE04	+	M	+	S	
CFJD132	+	W	M	M	
CFKD007	+	-	-	-	
CFJD023	+	M	W	S	
CFKD113	+	-	47.0°C	W	
CFLP83	+	-	-	-	
CFLP65	+	-	53.8°C	S	
CFFD093	+	51.4°C	51.4°C	-	
CFWP7	+	-	-	-	
CFZB111	+	-	-	W	
CFZB27	+	M	M	56.7°C	
CFZB07	+	W	W	46.7°C	
CFFD144	+	-	-	-	
CFSSR001	+	W	-	-	
CFMSM019	+	-	-	M	
CFMSM009	+	-	-	-	
CFMSP003	+	M	M	M	
CFMSP075	+	M	W	W	
CFMSM016	+	54°C	54°C	54°C	√
CFMSM020	+	49°C	49°C	49°C	√
CFMSP007	+	M	W	M	
CFMSM014	+	W	-	M	
CFMSM018	+	W	W	W	
CFAD243	+	-	W	-	
CFAD245	+	-	M	M	
CFBD119	+	+	+	-	
CFCD103	+	+	-	W	
CFAD157	+	-	-	-	
CFAD183	+	-	-	-	
CFAD068	+	-	-	-	
CFDD097	+	-	-	-	
CFAD259	+	-	-	-	
CFCD131	+	54.0°C	54°C	54°C	√
CFGD046	+	45.0°C	45.0°C	45°C	√
CFLP072	+	-	-	-	
CFLD063	+	-	-	-	
CFLD009	+	-	-	-	
CFFD158	+	-	-	-	
CFML06	+	-	-	-	
CFMD016	+	-	-	-	
CFMD013	+	-	-	-	
CFOD082	+	-	-	-	
CFFD172	+	-	-	-	
CFBD022	+	W	M	M	
CFBD204	+	-	-	-	
CFLD144	+	-	-	-	
CFAD006	+	-	-	-	
Total	72	11	12	12	
M. yessoensis loci					
XY106	-	+	S	-	
XY129	-	+	-	45.0°C	
PYER010	-	+	-	-	
PYER011	49.0°C	+	-	-	
PYER015	-	+	W	-	
PYER023	-	+	-	-	
PYER031	-	+	W	45.0°C	
PYMSM005	-	+	-	-	
XY138	-	+	-	-	
XY105	50°C	+	50.0°C	50.0°C	
XY111	M	+	M	M	
XY128	53.8°C	+	W	49.0°C	
Total	3	12	1	4	
C. nobilis loci					
CNER053	W	W	+	W	
CNER048	-	W	+	-	

Loci	Amplification results in four species				
	C. farreri	*M. yessoensis*	*C. nobilis*	*A. irradians*	Transferbility in four species
CNER036	W	W	+	-	
CNER064	M	M	+	-	
CNER067	-	M	+	-	
CNER003	-	-	+	-	
CNER041	-	-	+	-	
CNER021	W	53.8°C	+	53.8°C	
CNER070	M	W	+	53.8°C	
Total	0	1	9	2	
A. irradians loci					
AIMS023	51.4°C	-	-	+	
AIMS026	-	-	W	+	
AIMS027	M	M	W	+	
AIMS028	-	M	-	+	
AIMS021	M	-	-	+	
AIMS021	-	-	-	+	
AIMS022	W	W	W	+	
AIMS025	M	W	M	+	
AIMS027	M	45.4°C	M	+	
Total	1	1	0	9	

+ : amplified in the focal species; -: no product; M: unorderly bands; W: weak bands.

3.2. Polymorphism Evaluation of the Four Species Transferable Markers

Five markers (CFMSM016, CFMSM020, CFCD131, CFGD046 and CFE04) were found to be successfully transferred among the four species, while many were unique for one species which might be useful for species identification, such as CFAD019 only amplified in *C. farreri*. When the five marker's polymorphism in six individual of each species were detected, it was found that there were some individuals that can't be amplified in certain loci, which might be caused by null allele or other reasons. Only CFMSM016, CFCD131 and CFE04 could be amplified in all individuals. Their annealing temperature and allele numbers were listed below (Table 2).

Table 2. *The polymorphism information of the three markers in four scallop species.*

Loci		CFMSM016	CFCD131	CFE04
Annealing temperature		54°C	54°C	47°C
Allele number	*C. farreri*	3	3	4
	M. yessoensis	2	2	2
	C. nobilis	1	2	2
	A. irradians	1	1	2

4. Discussion

As shown in the results, fewer than 14.3% microsatellite markers are likely to be useful for related scallop species. Only three markers can be amplified in all four species, revealing low transferability rate of microsatellites from the family Pectinidae. It is in agreement with the result observed previously [16], in which they test cross amplification of microsatellites originating from *A. irradians* EST database in the four species. Another study also proclaims poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, with *C. gigas* versus *C. ariakensis* (36.0%), and *C. gigas* versus *C. virginica* (12.8%) [10]. A possible reason is that they have great genetic divergence and evolutionary time span. In addition, no amplification bias was found between the four scallop species.

However, PCR success may have been underestimated in non-focal species because of the optimization limitations in the experiments. Only limited PCR reactions and gels have been run for each primer set, and comparisons have been conducted mainly under the PCR conditions optimized for the focal species. Despite these factors, a clear signal of PCR decay has been observed. In addition, decline in the ability to amplify microsatellites from related species is paralleled by a decline in allelic diversity for those markers that do amplify (Table 2). However, we expect this research can benefit new microsatellites markers isolation in related species of scallop and be helpful for ecological investigation, phylogenetic study and even species identification of pectinids.

5. Conclusions

In this study, it revealed low transferability of microsatellites from scallops of the family Pectinidae. Three out of 103 microsatellite markers (CFMS016, CFCD131 and CFE04) were successfully amplified across four scallop species (*A. irradians, C. farreri, C. nobilis, and M. yessoensis*) and polymorphic. Most of the other markers showed poor cross-species amplification, among which certain markers were specific to one species. These markers are expected to supply valuable genetic sources for ecological investigation and species identification of scallops.

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