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Microchip Electrophoresis System MultiNA™ II MCE-301

Application News

Identification of Thunnus Species by PCR-RFLP Method Using MultiNA II

Shinichiro Kobayashi

User Benefits

- Since the electrophoresis operation is fully automated, the processes from sample preparation to detection can be largely simplified.
- High analytical performance and reproducibility are achieved by selecting the optimal separation buffer and internal maker for each sample.
- Fingerprinting analysis enables automatic judgment of the presence of DNA fragments in samples.

■ Introduction

For seafood distributed in Japan, a quality labeling standard system has been established under the Act on Japanese Agricultural Standards (Act on Standardization and Proper Quality Labeling of Agricultural and Forestry Products), which requires accurate display of items such as the food product name and place of origin. Although the Japanese consume large amounts of fish belonging to the genus *Thunnus* (tuna), it is difficult to identify the various varieties of tuna from the external appearance in the fresh or processed condition. For this reason, misidentification, inaccurate labeling, and misrepresentation of the fish type in the distribution process have taken up social problems, requiring a quick, simple and accurate technology for distinguishing fish varieties. This article introduces an example of identification of the tuna varieties of Atlantic bluefin tuna (*Thunnus thynnus*), southern bluefin tuna (*T. maccoyii*), α-type and β-type bigeye tuna (*T. obesus*), yellowfin tuna (*T. albacares*), and albacore tuna (*T. alalunga*) using the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method established by Japan's Food and Agricultural Materials Inspection Center (FAMIC). In this article, a MultiNA II MCE-301 microchip electrophoresis system (Fig. 1) was employed to detect the separation patterns of the PCR-RFLP products used to distinguish the fish varieties.

■ Sample Preparation and Analysis Conditions

The DNA extraction and PCR conditions were referred to the literatures[1)-5)] (Fig. 2). DNA was extracted from samples of the flesh of Atlantic bluefin tuna, southern bluefin tuna, α and β-type bigeye tuna, yellowfin tuna, and albacore tuna.

Using the DNA extracted from each type of tuna as templates, PCR was conducted with primers specific to the mitochondrial DNA of each tuna, and the obtained PCR products were processed with restriction enzymes (*Alu* I, *Mse* I, *Tsp*509 I). Electrophoresis of the digest fragments obtained with the various restriction enzymes was carried out using the MultiNA II, and the fish varieties were distinguished based on the differences in the electrophoresis patterns of the fragments.

■ Analysis Conditions for PCR-RFLP Products

Instrument : MultiNA™ II MCE-301

Analysis mode : DNA-500 on-chip mode

Fig. 1 MultiNA™ II MCE-301 Microchip Electrophoresis System

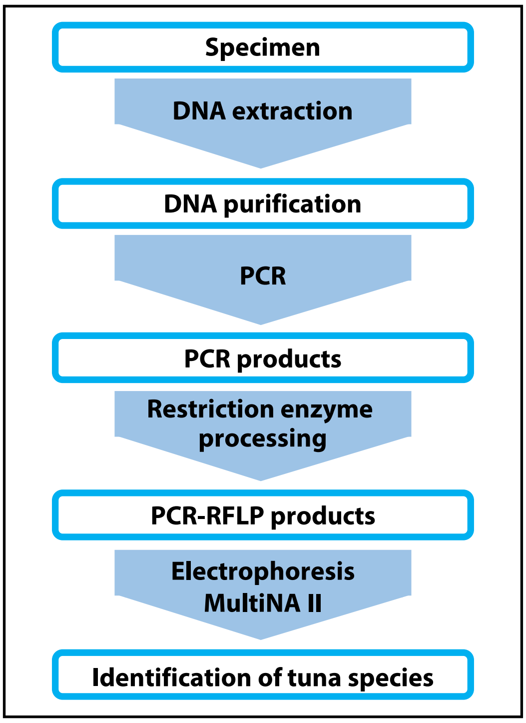

Fig. 2 Procedure for Identification of Tuna Species by PCR-RFLP Method

■ Results

Fig. 3 shows the results of the analysis of the PCR-RFLP products of Atlantic bluefin tuna (*T. thynnus*), southern bluefin tuna (*T. maccoyii*), α-type and β-type bigeye tuna (*T. obesus*), yellowfin tuna (*T. albacares*), and albacore tuna (*T. alalunga*) by the MultiNA II. Atlantic bluefin tuna, β-type bigeye tuna, and albacore tuna showed distinctive fragmentation patterns as a result of restriction enzyme processing with *Alu* I, making it possible to distinguish these varieties (in Fig. 3, *Alu* I processing indicated by the ★ symbol). However, southern bluefin tuna, α-type bigeye tuna, and yellowfin tuna showed the same fragment patterns. Next, restriction enzyme processing using *Mse* I was carried out. Southern bluefin tuna showed a distinctive fragmentation pattern with restriction enzyme processing using *Mse* I, allowing its identification (in Fig. 3, *Mse* I processing indicated by the ★ symbol). Therefore, restriction enzyme processing of the remaining two varieties, α-type bigeye tuna and yellowfin tuna, was conducted with *Tsp*509 I, and the two varieties could be distinguished by the resulting fragmentation patterns (in Fig. 3, *Tsp*509 I processing indicated by the ★ symbol). Furthermore, when a positive control exists, the presence or absence of each DNA fragment in the sample can be judged automatically by performing the fingerprinting analysis. After the analysis, the results can be displayed in a list form. Here, the PCR-RFLP products obtained by *Alu* I and *Mse* I processing of the unknown sample were judged to be southern bluefin tuna based on the patterns (see Fig. 3 and Table 1).

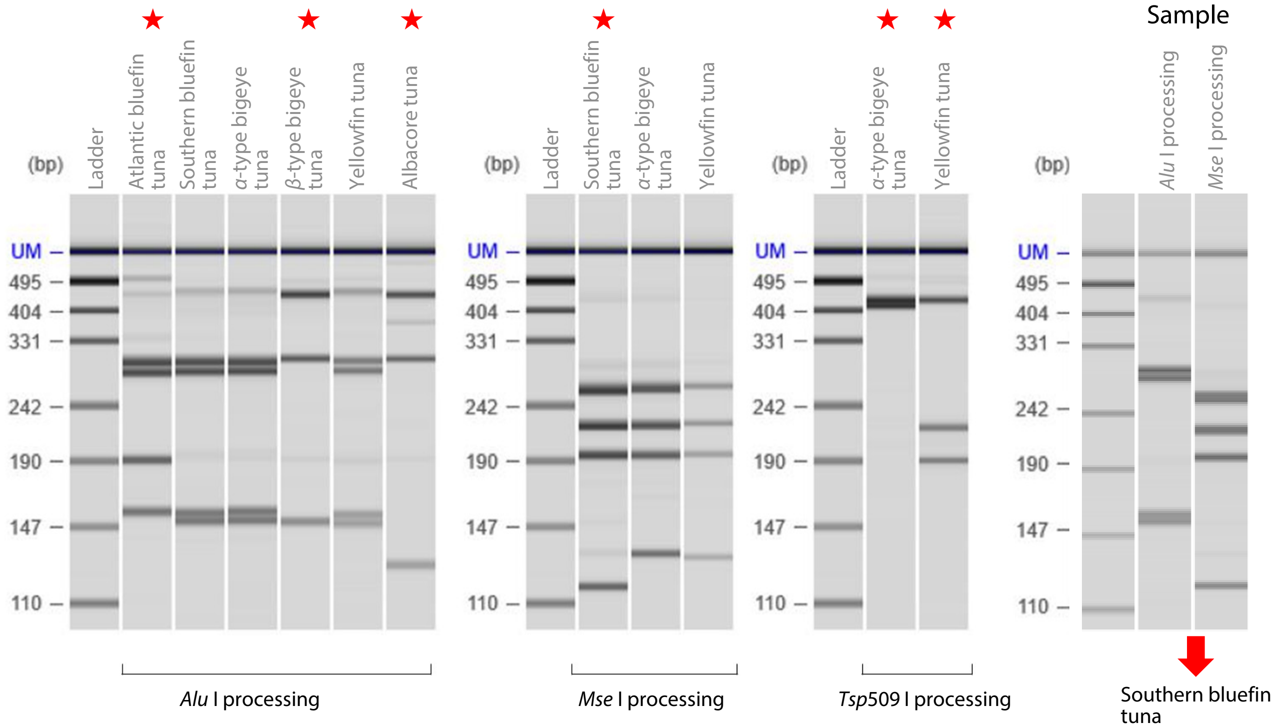

Fig. 3 PCR-RFLP Patterns of Tuna Species

Table 1 Judgment Patterns of Tuna Species

	Alu I processing								*Mse* I processing					*Tsp*509 I processing	
Variety	Atlantic bluefin tuna	Southern bluefin tuna	α-type bigeye tuna	β-type bigeye tuna	Yellowfin tuna	Albacore tuna	Sample	Variety	Southern bluefin tuna	α-type bigeye tuna	Yellowfin tuna	Sample	Variety	α-type bigeye tuna	Yellowfin tuna
298 bp	+	+	+	+	+	+	+	268 bp	+	+	+	+	429 bp	+	+
284 bp	+	+	+	-	+	-	+	220 bp	+	+	+	+	413 bp	+	-
190 bp	+	-	-	-	-	-	-	194 bp	+	+	+	+	218 bp	-	+
155 bp	+	+	+	-	+	-	+	133 bp	-	+	+	-	190 bp	-	+
148 bp	-	+	+	+	+	-	+	118 bp	+	-	-	+			

<References>

1) Chow S.: Bull. Natl. Res. Inst. Far Seas Fish., 30, 619-627 (1993)
2) Chow S.: J. Mol. Evol., 41,741-748 (1995)
3) Chow S.: Mol. Eco., 9,221-227 (2000)
4) Takeyama H.: J. Fish Biology, 58, 1646-1657 (2001)
5) Chow S.: Bull. Fish. Res. Agen., 8, 1-14 (2003)

■ Conclusion

Food product identification of tuna species was possible by the PCR-RFLP method using the MultiNA II microchip electrophoresis system. MultiNA II can automatically analyze reagents and samples by simply setting them to the tray. In addition, the fingerprinting analysis can recognize DNA fragments in each sample and identify species from the electrophoresis patterns of PCR-RFLP products.

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