LAAN-A-SP-E006

SHIMADZU

Application News

Microchip Electrophoresis No.B28

Identification of Thunnus Using PCR-RFLP Method with MCE-202 "MultiNA"

Consumer concern over food safety has steadily risen in recent years. Responding to this concern, the Japanese Agricultural Standard (Law Concerning Standardization and Proper Labeling of Agricultural and Forestry Products) was revised. The Quality Labeling Standard System was established, requiring the clear and accurate display of food product name, country of origin, etc. It is the responsibility of the manufacturer or the distributor to accurately convey this information as a product selection guideline to consumers.
For example, seafood belonging to the tuna species, which is consumed in large quantities by the Japanese, is difficult to distinguish among the various types when presented in the fresh or processed seafood state. Therefore, misidentification, inaccurate labeling and disguise during the distribution process are considered to be social problems, therefore requiring a technique that can quickly, simply and accurately distinguish among product varieties.
Here we introduce the procedure for distinguishing the differences among fish stock of Atlantic bluefin tuna (Thunnus thynnus), southern bluefin tuna (T. maccoyii), α and β 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 as described in the manual produced by the Food and Agricultural Materials Inspection Center and the National Research Institute of Fisheries Science, Fisheries Research Agency. The MCE-202 "MultiNA" microchip electrophoresis analyzer was used for detection of the separation patterns of the PCR-RFLP products used for distinguishing the differences between these types of fish stocks.

■ Experimental Procedure

The DNA extraction and PCR conditions conformed to those presented in the Manual for Distinguishing Among Tuna Fish Stocks* produced by the Food and Agricultural Materials Inspection Center and the National Research Institute of Fisheries Science, Fisheries Research Agency. DNA was extracted from pieces of Atlantic bluefin tuna, southern bluefin tuna, α and β bigeye tuna, yellowfin tuna and albacore tuna.
PCR was conducted using the DNA extracted from the various types of tuna as templates. Primers specific to the tuna mitochondrial DNA were used for PCR amplification. The obtained PCR products were processed using restriction enzymes (Alu I, Mse I, Tsp509 I). Electrophoresis of the obtained enzyme digest fragments was conducted using the MultiNA, and the fish varieties were distinguished based on the differences in the fragment patterns.

(*)Manual for Identification of Thunnus Species
Food and Agricultural Materials Inspection Center, National Research Institute of Fisheries Science, Fisheries Research Agency
http://www.famic.go.jp/technical_information/hinpyou/pdf/maguro_manual.pdf
(Japanese)

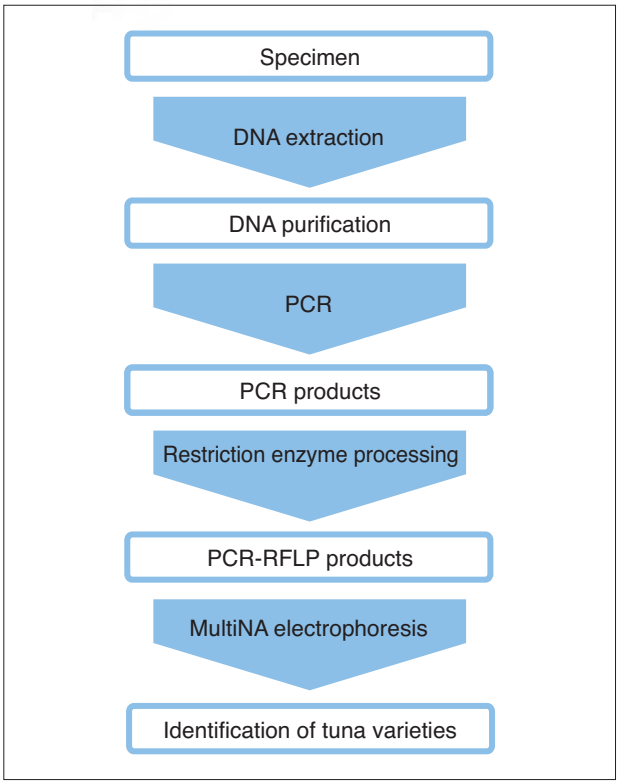

Fig. 1 Experimental Procedure of Identification of Thunnus Using PCR-RFLP Method

■ Reagents / Kits

·DNA-500 Kit
(Shimadzu)
·SYBR® Gold nucleic acid gel stain
(Invitrogen)
·25 bp DNA Ladder
(Invitrogen)
·DNeasy Blood & Tissue Kit
(Qiagen)
·*Alu* I
(New England Biolabs Japan)
R0137S
·*Mse* I
(New England Biolabs Japan)
R0525S
·*Tsp*509 I
(New England Biolabs Japan)
R0576S

■ Analytical Conditions for PCR-RFLP Products

Instrument : MCE-202 "MultiNA"
Analysis Mode : DNA-500 on-chip mode

■ Results

Fig. 2 shows the results of analysis of the PCR-RFLP products from Atlantic bluefin tuna (Thunnus thynnus), southern bluefin tuna (T. maccoyii), α and β bigeye tuna (T. obesus), yellowfin tuna (T. albacares), and albacore tuna (T. alalunga) by MultiNA.
The Atlantic bluefin tuna, β bigeye tuna, and albacore tuna show distinctive fragment patterns as a result of *Alu* I restriction enzyme processing, allowing them to be distinguished (*Alu* I processing marked with★ in Fig. 2).
However, the southern bluefin tuna, α bigeye tuna, and yellowfin tuna show the same fragmentation pattern. Therefore, we next performed restriction enzyme processing using Mse I. The southern bluefin tuna showed a distinct fragmentation pattern as a result of restriction enzyme processing using *Mse* I, allowing its identification (*Mse* I processing marked with★ in Fig. 2).
As for the remaining α bigeye tuna and yellowfin tuna, 2 types of distinct fragmentation patterns were observed as a result of *Tsp* 509 I restriction enzyme processing, allowing these to be easily distinguished (*Tsp* 509 I processing marked with★ in Fig. 2).
The excellent sensitivity, separation and repeatability of the analysis data obtained with the MultiNA demonstrate that it is a powerful and fully automated tool for determining intra-species genetic variation.

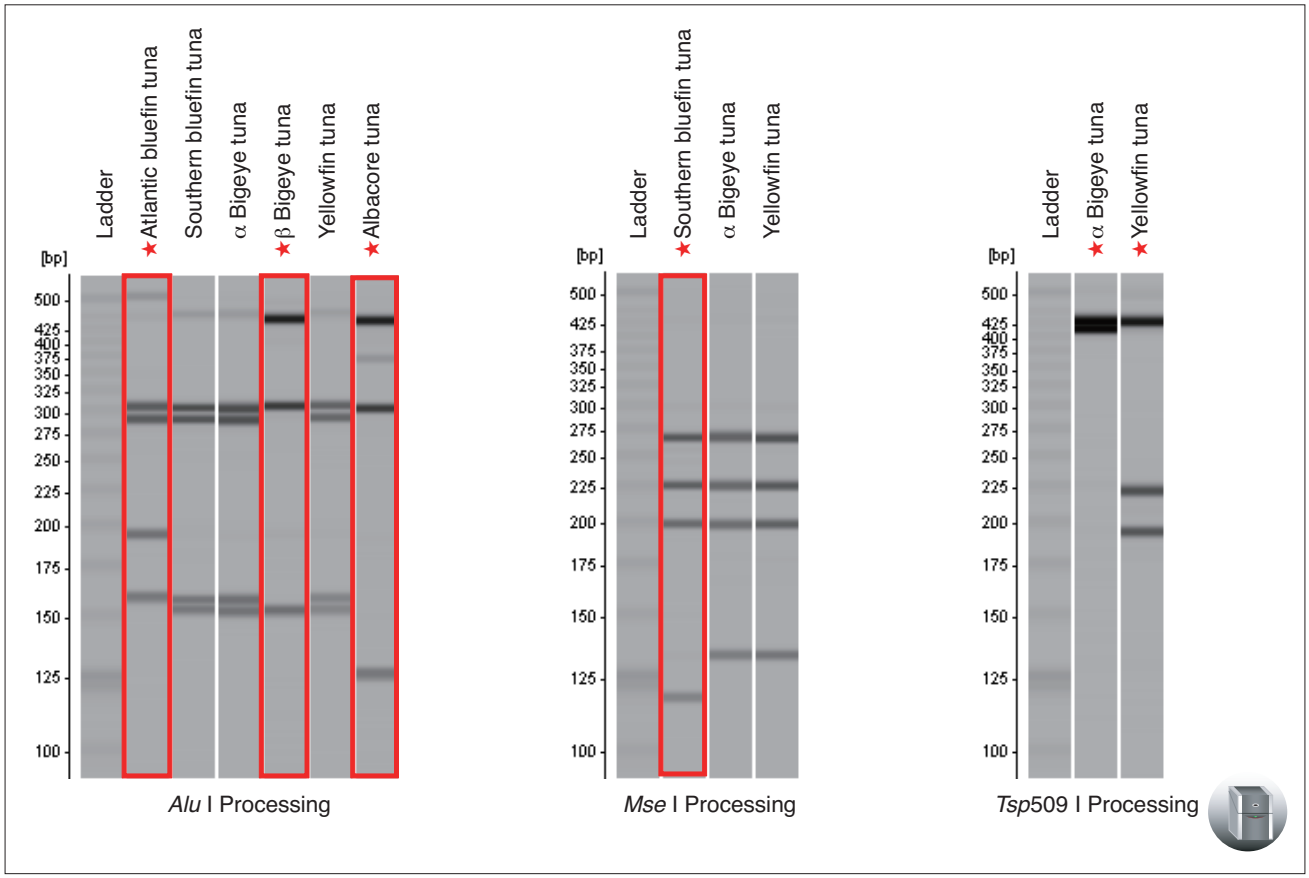

Fig. 2 Analytical Results for PCR-RFLP Products from Thunnus

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