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Short communication

Development of primer set for the identification of fish species in surimi products using denaturing gradient gel electrophoresis

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ABSTRACT

The purpose of this study was to develop a DNA marker for the identification of fish species in processed surimi products. A DNA marker was designed based on the mitochondrial cytochrome c oxidase subunit I gene, and fish species in surimi products were identified using a molecular fingerprinting technique, denaturing gradient gel electrophoresis (DGGE); the results were subjected to sequence-based analysis. The DGGE profiles indicated the presence in surimi products of a greater diversity of fish species than reported previously: 20 species belonging to 16 genera were identified. Therefore, our method facilitates the simple and rapid detection and identification of fish species in seafood products produced from minced fish.

1. Introduction

Surimi is a processed seafood that is simple to prepare and low cost (Yin & Park, 2014; Yoon, Kim, Kim, Jo, & Cho, 2014). Imitation crab meat, which is made from white fish, such as pollock and cod, is an example of a surimi product. Alaska pollock (*Theragra chalcogramma*) is a major raw material for surimi (Poowakanjana & Park, 2013). Because of the decline in the Alaska pollock catch rate from 250,000 MT in 2003 to about 125,000 MT in 2010, other fish species have been considered for surimi production (Poowakanjana & Park, 2013). Consequently, Pacific whiting (*Merluccius productus*), northern blue whiting (*Micromesistius poutassou*), southern Blue whiting (*Micromesistius australis*), atka mackerel (*Pleurogrammus azonus*), threadfin bream (*Nemipterus sp.*) and jack mackerel (*Trachurus murphy*) are now in use as raw materials for production of surimi (Park, 2005).

Food companies and consumers are focused on the safety and quality of food, and surimi quality is influenced by the type of fish included (Shiku, Hamaguchi, Benjakul, Visessanguan, & Tanaka, 2004). In general, whiteness and texture of white flesh fish result in a high-quality product with high-protein and low-fat (Martin-Sanchez, Navarro, Perez-Alvarez, & Kuri, 2009). Therefore, identifying the fish species in surimi is important for quality assurance. Some companies seek to make a profit by replacing higher-priced with lower-priced fish (Keskin & Atar, 2012). Indeed, substituting expensive fish species with those of lower cost is easy to use for unfair profits and illegal sales such as fraudulent labeling because consumers are unable to identify the fish species in surimi products (Huxley-Jones, Shaw, Fletcher, & Parnell, 2012). A study of fish fillets reported that lower-cost fish species were used in place of those specified on the label (Pinto et al., 2015). According to the U.S. Food and Drug Administration and the European Union guidelines, the most important factor for seafood quality control is identification of the fish species therein (Galal-Khallaf, Ardura, Borrell, & Garcia-Vazquez, 2016; Keskin & Atar, 2012). Rapid and accurate identification of fish species is, therefore, essential. Additionally, the profit motive and rapid increases in demand have resulted in overfishing and in various fish species becoming endangered (Galal-Khallaf et al., 2016). Therefore, the identification of the fish species in surimi is required for the management of overfishing and the conservation of endangered species.

DNA-based analysis is required for the identification of fish

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species in surimi, as it is virtually impossible to distinguish fish species based on their morphology (Huxley-Jones et al., 2012). Several recent studies have employed molecular techniques to identify the fish species in processed seafood products (Galal-Khallaf et al., 2016; Keskin & Atar, 2012; Pinto et al., 2015; Zhao et al., 2013). However, because those studies were focused on identifying only a single species, the methods are unsuitable for use with surimi.

Denaturing gradient gel electrophoresis (DGGE) uses the reduction in electrophoretic mobility according to the denaturing characteristics of PCR products to facilitate their in-gel separation (Muyzer & Smalla, 1998). Therefore, DGGE enables the identification of the various fish species in a minced fish product (Boon, Windt, Verstraet, & Top, 2002; Muyzer, Waal, & Uitterlinden, 1993). DGGE analysis has been adapted by environmental microbiologists for bacterial community characterization (Griffiths, Whiteley, O'Donnell, & Bailey, 2000; Muyzer, 1999). This method has also been widely used to characterize microbial community diversity and composition as well as structural changes in various environments, including foodstuffs (Arcuri, Sheikha, Rychlik, Piro-Metayer, & Montet, 2013; Ercolini, 2004; Hong, Pruden, & Reardon, 2007). However, to our knowledge, no study has applied DGGE to identify the fish species in a minced foodstuff.

The aims of the present study were to design DGGE primers targeting the cytochrome c oxidase subunit I (COI) gene to identify the fish species in surimi products. To our knowledge, this study is the first attempt to analyze fish species using a DGGE method.

2. Materials and methods

2.1. Sample preparation

Twenty surimi products were purchased from markets in Busan, South Korea in February 2015 and transported to the laboratory under refrigerated conditions. Sample information is provided in Supplementary Table 1.

2.2. DNA extraction

Dried surimi product (200 mg) was subjected to DNA extraction in triplicate using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, California) according to the manufacturer's instructions. DNA was quantified using the NanoVue system (GE Healthcare Europe, Munich, Germany), and triplicate samples were pooled into a single sample.

2.3. Primer design and optimization

Whole-length mitochondrial sequences from five fish species used in surimi products—*Nemipterus virgatus* (KR701906), *Theragra chalcogramma* (AB094061), *Micromesistius poutassou* (FR751401), *Pleurogrammus azonus* (AB744047), and *Trachurus japonicus* (AP003092)—were obtained from the GenBank database at the NCBI (http://www.ncbi.nlm.gov) and were aligned using the ClustalW software in the BioEdit platform version 7.0.9.0 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html, Hall 1999) (Table 1). The COI gene was used as the target for identification of fish species. Design of the primers took into consideration the GC content, nucleotide composition at the 3′ end, melting temperature, secondary structure, product size, and coverage of fish species. A primer pair targeting a 213-bp region of the COI gene was designed (Table 2). Gradient PCR was performed at 46, 48, 50, 52, 54, and 56 °C to identify the optimum amplification temperature. The PCR products were separated in a 1% agarose gel and visualized using 1 × Redsafe Nucleic Acid Staining Solution (iNtRON, South Korea).

2.4. Primer test

To evaluate the coverage of the primer pair, 152 genomic DNA samples from 76 fish species of 28 families were obtained from the Fisheries' Genetic Resources Management Center (National Institute of Fisheries Science, South Korea); these are presented in the Supplementary Table 2. The reference sequences were aligned and compared with those of the ShortFish-F and ShortFish-R primers. Reference DNA was amplified by PCR with the designed primers and a 46–56 °C temperature gradient. A neighbor-joining tree was constructed using the MEGA 5.0 software to evaluate the resolution of the 213-bp amplicons.

2.5. DGGE-PCR amplification

To increase primer specificity, the touchdown PCR method was used. A GC-clamp (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG

Table 2
Universal primers designed from conserved region of COI gene.

Primer name	Sequence	Fragment size	Developed for
ShortFish-F	5′- CACAAAGACATTGGCACCC -3′	213 bp	Fish
ShortFish-R	5′- AGTCAGTTTCCGAACCCTCC -3′		

Table 1
Alignment of primer regions with complete mitochondrial sequences of reference species.

	5 6 4 1	5 6 4 2	5 6 4 3	5 6 4 4	5 6 4 5	5 6 4 6	5 6 4 7	5 6 4 8	5 6 4 9	5 6 5 0	5 6 5 1	5 6 5 2	5 6 5 3	5 6 5 4	5 6 5 5	5 6 5 6	5 6 5 7	5 6 5 8	5 6 5 9	
N. virgatus	**C**	**A**	**C**	**A**	**A**	**A**	**G**	**A**	**C**	**A**	**T**	**C**	**G**	**G**	**C**	**A**	**C**	**C**	**C**	
T. chalcogramma	**C**	**A**	**C**	**A**	**A**	**A**	**G**	**A**	**C**	**A**	**T**	**T**	**G**	**G**	**C**	**A**	**C**	**C**	**C**	
M. poutassou	**C**	**A**	**C**	**A**	**A**	**A**	**G**	**A**	**C**	**A**	**T**	**T**	**G**	**G**	**C**	**A**	**C**	**C**	**C**	
P. azonus	**C**	**A**	**C**	**A**	**A**	**A**	**G**	**A**	**C**	**A**	**T**	**T**	**G**	**G**	**C**	**A**	**C**	**C**	**C**	
T. japonicus	**C**	**A**	**C**	**A**	**A**	**A**	**G**	**A**	**C**	**A**	**T**	**C**	**G**	**G**	**C**	**A**	**C**	**C**	**C**	
	5 8 3 4	5 8 3 5	5 8 3 6	5 8 3 7	5 8 3 8	5 8 3 9	5 8 4 0	5 8 4 1	5 8 4 2	5 8 4 3	5 8 4 4	5 8 4 5	5 8 4 6	5 8 4 7	5 8 4 8	5 8 4 9	5 8 5 0	5 8 5 1	5 8 5 2	5 8 5 3
N. virgatus	**G**	**G**	**C**	**G**	**G**	**G**	**T**	**T**	**C**	**G**	**G**	**A**	**A**	**A**	**C**	**T**	**G**	**A**	**C**	**T**
T. chalcogramma	**G**	**G**	**A**	**G**	**G**	**C**	**T**	**T**	**T**	**G**	**G**	**A**	**A**	**A**	**C**	**T**	**G**	**A**	**C**	**T**
M. poutassou	**G**	**G**	**C**	**G**	**G**	**C**	**T**	**T**	**C**	**G**	**G**	**G**	**A**	**A**	**C**	**T**	**G**	**A**	**C**	**T**
P. azonus	**G**	**G**	**C**	**G**	**G**	**T**	**T**	**T**	**C**	**G**	**G**	**G**	**A**	**A**	**C**	**T**	**G**	**A**	**C**	**T**
T. japonicus	**G**	**G**	**A**	**G**	**G**	**C**	**T**	**T**	**T**	**G**	**G**	**A**	**A**	**A**	**C**	**T**	**G**	**A**	**C**	**T**