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THE SPOILAGE BACTERIA IN FISH PRODUCTS HAMPER GENETIC IDENTIFICATION OF FISH SPECIES VIA COI SEQUENCING

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The mislabeling of fish and fish products quite often occurs worldwide. The fish substitution in food can cause different consequences varying from economic losses to adverse impact on consumers' health. Nowadays the partial sequence of COI gene is used successfully for genetic identification of fish species in food. The standard procedure of genetic identification includes PCR amplification of partial COI gene followed by Sanger sequencing [1]. However, fish is a perishable food and fish spoilage can occur before entering laboratory. The standard procedure for genetic identification did not work well in these cases. The next generation sequencing of problematic samples showed that PCR amplicons contained sequences corresponding to COI gene of fish and typical spoilage bacteria (Pseudomonas spp., Comamonas spp., Shewanella spp., Delftia spp.). The alignment of DNA sequences showed that COI genes of fish and some spoilage bacteria had enough homology to allow efficient annealing of universal COI primers. To avoid PCR amplification of bacterial DNA we choose other universal forward primer located upstream of the COI gene. However, the sequence flanked by novel pair of primers contained the GC-rich region, so PCR were not robust enough and required the use of special PCR mix. DNA pretreatment with BstUI endonuclease for selective restriction of bacterial DNA was useful only in cases of low bacterial contamination. The bacterial contamination did not affect analysis when 16S rRNA gene used for genetic identification of fish. In our experience, PCR of partial 16S rRNA gene generated only fish specific amplicons even upon severe bacterial contamination. However, Sanger sequencing of 16S rRNA gene allowed us to identify some fish samples only at the genus level.

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References:

[1] Handy S. M. et al. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. Journal of AOAC International, 2011, 94 (1), 201-210