MULTIPLE DNA BARCODING FOR FISH SPECIES IDENTIFICATION IN SUSHI PRODUCTS

Lara Tinacci[1] Alessandra Guidi[1] Lorenzo Castigliego[1] Raniero Lorenzetti[2] Daniela Gianfaldoni[1] and Andrea Armani[1]

[1]Department of Veterinary Science, University of Pisa

[2]IZS Lazio e Toscana

The aim of this work was to perform a molecular survey based on DNA barcoding to identify the seafood species used in the preparation of ethnic products (sushi). Twenty-one raw products (each composed of 3 to 8 pieces, for a total of 88 samples) were purchased in ethnic restaurants in the provinces of Pisa (11), Lucca (2), Livorno (3) and Florence (5). The total DNA extracted (1) was evaluated by gel electrophoresis and amplified using universal primers for mitochondrial (COI, 16SrRNA) or nuclear genes (PEPCK) depending on the species (fish, mollusk or crustacean) and the level of DNA degradation. Different primers (2,3,4,5,6,7) for the amplification of a long (~700 bp) or a short (~139-200 bp) fragment were used. Ninety-five PCR products were obtained (for some products two genes were analyzed). Of these, 30 have already been sequenced (Experimental Zooprophylactic Institute of Latium and Tuscany (Rome)). The sequences were elaborated with Clustal W in Bioedit 7.0.9.0, and analyzed by a BLAST analysis on GenBank and by using the Identification System on BOLD. A top match with a sequence similarity of at least 98% was used to designate potential species identification (8). DNA was degraded in almost one third of the samples. This was probably due to rice acidification, to repeated cycles of freezing/thawing or to prolonged storage. The degradation was confirmed by PCR amplification. In fact, we obtained long amplicons in 72.6% of the cases (n=69) and short amplicons for 27.3% of the samples (n=26). The average length of the long sequences was 595 bp for the COI FDB and 490 bp for the PEPCK gene, while the length of the short sequences was ~210bp for the 16S rRNA and 139bp for the COI MDB. All the samples were identified at least at the genus level, with identity values ranging from 99 to 100%. Although for some samples it was impossible to achieve a specific identification, the results were informative enough to verify the information given by the producers. No samples were found mislabeled. Even though the COI gene represents the most exploited target for seafood species identification, issues were found during amplification and comparison with the databases. Thus, in order to increase the PCR output, new universal primers, able to amplify a wide range of taxa, would be desirable. Finally, in case of degraded DNA samples, where the number of diagnostic mutation is limited, a multiple gene analysis is advisable.

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