A higher sensitivity and efficiency of common primer multiplex PCR assay in identification of meat origin using NADH dehydrogenase subunit 4 gene

ABSTRACT

A Common Primer Multiplex PCR (CP-M-PCR) was developed to detect meat origin of four groups of animal (pig, ruminant, avian and rabbit). This method demonstrated higher sensitivity and efficiency than the conventional multiplex PCR. In this approach, a common forward primer was designed in the 5′ end of a homologous region of mitochondrial NADH dehyrogenase subunit 4 (Nad 4) gene sequences of all the animal groups. Specific adapter reverse primers were designed by adding an adapter sequence at the 5′ end. The same adapter sequence was used as the common adapter reverse primer. The primers generated specific fragments of 267, 370, 504, and 548 bp lengths for pig, ruminant, avian and rabbit meats, respectively. The use of adapter sequence at the 5′ end of the common adapter reverse primers increased the efficiency of the amplification and the application of a common forward primer solved the complexity in multiplex PCR system. Bands of specific amplification can be detected in the PCR assays containing as low as 10−6 μM of adapter reverse primer. This result indicated that the sensitivity was tremendously increased as compared to the conventional multiplex PCR (10−3 μM). CP-M-PCR detection limit of the DNA samples was 0.1 ng for the four groups of meats. CP-M-PCR has greatly improved the sensitivity and efficiency of the PCR system for a more reliable and accurate outcome than conventional multiplex PCR system.

Keyword: Multiplex; Nad 4; Meat; Common primer