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Short-length DNA marker for the determination of Malayan box turtle (*Cuora amboinensis*) materials in food chain and traditional Chinese medicines

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Malayan box turtle (*Cuora amboinensis*) (MBT) is a protected species and prohibited in Muslim foods and medicines. Despite having medicinal values, turtles are reservoirs of heavy metals and potential carriers of health-threatening microbes and allergens. To monitor turtle traffcking, there is a need of a convenient and reliable method for the quantitative tracing of turtle materials in food chain and medicines. Several polymerase chain reaction (PCR) assays have been proposed for the detection of MBT species under various routes but they are based on long-length targets which break down under the state of decomposition, making them unsuitable for the forensic and archaeological detection in food chain, medicines and other potential routes. To overcome this research gap, for the first time, we developed and validated a short length DNA marker for the qualitative and quantitative detection of MBT tissues by Conventional PCR, PCR-RFLP and SYBR green real-time PCR systems. It combined a 120 bp-site of the MBT mitochondrial cytochrome b gene and a 141bp-site of 18S rRNA gene as the universal marker for the eukaryotes. The assay specificity was checked against 20 different species and biomarker stability was tested under various food processing conditions, including boiling, autoclaving and micro oven heating under pure, admixed and commercial food matrices. The limit of detection (LOD) of the conventional PCR and PCR-RFLP assays was 0.0001 ng MBT DNA under pure state and 0.01% (w/w) MBT meat under admixed and commercial matrices. In contrast, the LOD of the SYBR green duplex PCR system was 0.00001 ng DNA and 0.001% (w/w) MBT meat under mixed matrices. PCR amplified target was further validated by sequencing and restriction digestion with *Bfa*1 endonuclease and distinctive fingerprints (72, 43 and 5 bp) were obtained. The MBT target was further quantified by a duplex SYBR green real time PCR system consisting of MBT target and internal positive control, wherein the melting curve clearly reflected two distinctive peaks at 74.63±0.22°C and 81.40±0.31°C for the MBT and eukaryotic targets, respectively, under pure, admixed and commercial matrices. The quantification limit (ng) was 0.00001for pure meat, 0.0030±0.00001 for binary mixtures, 0.0021±0.00008 for meatball, 0.0042±0.0037 burger and 0.0013±0.00006 frankfurter products. The analysis of 150 reference meat samples reflected 98.19 to 166.57 % target recovery, 92.23-98.15 % PCR efficiency and 0.001% LOD under various matrices. A total of 183 commercial meat products were screened but no turtle contamination was found. Finally, 153 and 120 TCM samples were surveyed by PCR-RFLP and SYBR Green PCR and 40% and 23% of them were found to be MBT-positive (0.00157 to 0.0612 ng/µL), respectively. These authentications provided better security, firstly, through short-length biomarker target which offer extraordinary stability and sensitivity and secondly, through molecular fingerprints which authenticated the post-amplified target by restriction digestion. Thus, the novel assay demonstrated sufficient merit for use in any forensic and/or archaeological authentication of MBT, even under a state of decomposition.

Biography

Asing is a graduate student at University of Malaya, Malaysia and he is working under the supervision of Dr Md Eaqub Ali.

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