

Swine-Specific Mitochondrial Semiquantitative PCR-RFLP Assay Targeting Cytochrome B Gene for Detection of Pork in Commercial Meat Products

Abstract

Verification of pork adulteration in commercial meat products is increasingly important for the authentication of Halal labels in processed foods. Here, we documented a PCR-restriction fragment length polymorphism (RFLP) assay with high precision and reproducibility for the tracing of porcine DNA in commercial meat products. The assay combined the species-specific primers to selectively amplify a short fragment (109 bp) of porcine cytochrome b gene from a heterogeneous background of genomic DNAs followed by RFLP analysis to authenticate real amplicon. The analysis of PCR products and restriction digests was automated in a chip-based capillary electrophoresis incorporated in Agilent 2100 bioanalyzer. The swine specificity of the assay was checked with 11 different meat-providing animal and fish species. Model experiments, mimicking the processed foods, were performed in binary and ternary mixtures after mechanical grinding and prolonged autoclaving. Finally, four types of the most popular finished meat products (meatball, streaky bacon, frankfurter, and burger) which are prevalent in the Malaysian food market were analyzed in order to verify the assay performance. The assay was sensitive enough to detect 0.0001 ng of swine DNA in pure formats and 0.01% (w/w) spiked pork in extensively processed ternary mixture of pork, beef, and wheat flour.