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Detection of Aflatoxin B1 Producing Aspergillus flavus Genes from Maize Feed Using Loop-Mediated Isothermal Amplification (LAMP) Technique

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Abstract : Aflatoxin contamination in maize, one of several agriculture crops grown for livestock feeding, is still a problem throughout the world mainly under hot and humid weather conditions like Thailand. In this study Aspergillus flavus (A. Flavus), the key fungus for aflatoxin production especially aflatoxin B1 (AFB1), isolated from naturally infected maize were identified and characterized according to colony morphology and PCR using ITS, Beta-tubulin and calmodulin genes. The strains were analysed for the presence of four aflatoxigenic biosynthesis genes in relation to their capability to produce AFB1, Ver1, Omt1, Nor1, and aflR. Aflatoxin production was then confirmed using immunoaffinity column technique. A loop-mediated isothermal amplification (LAMP) was applied as an innovative technique for rapid detection of target nucleic acid. The reaction condition was optimized at 65□C for 60 min. and calcein flurescent reagent was added before amplification. The LAMP results showed clear differences between positive and negative reactions in end point analysis under daylight and UV light by the naked eye. In daylight, the samples with AFB1 producing A. Flavus genes developed a yellow to green color, but those without the genes retained the orange color. When excited with UV light, the positive samples become visible by bright green fluorescence. LAMP reactions were positive after addition of purified target DNA until dilutions of 10^{-6}. The reaction products were then confirmed and visualized with 1% agarose gel electrophoresis. In this regards, 50 maize samples were collected from dairy farms and tested for the presence of four aflatoxigenic biosynthesis genes using LAMP technique. The results were positive in 18 samples (36%) but negative in 32 samples (64%). All of the samples were rechecked by PCR and the results were the same as LAMP, indicating 100% specificity. Additionally, when compared with the immunoaffinity column-based aflatoxin analysis, there was a significant correlation between LAMP results and aflatoxin analysis ($r = 0.83$, $P < 0.05$) which suggested that positive maize samples were likely to be a high- risk feed. In conclusion, the LAMP developed in this study can provide a simple and rapid approach for detecting AFB1 producing A. Flavus genes from maize and appeared to be a promising tool for the prediction of potential aflatoxigenic risk in livestock feedings.

Keywords : Aflatoxin B1, Aspergillus flavus genes, maize, loop-mediated isothermal amplification
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