Quantification of Ochratoxin A–Producing Fungi in Coffee Products Using Quantitative PCR

Jakapan Potipun, Wijittra Ruaylarb, Rungrote Nilthong and Amorn Owatworakit*

School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

**Corresponding author. E-mail: amorn@mfu.ac.th*

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ABSTRACT

Ochratoxin A (OTA) is a polyketide mycotoxin that is produced by Aspergillus and Penicillium. Food contaminated with OTA poses health risks and is a food-safety challenge. Quantitative polymerase chain reaction (qPCR) has been used to identify non-toxigenic and toxigenic strains from coffee samples using polyketide synthase (pks), the OTA synthesis gene. In this research, Aspergillus carbonarius (ochratoxin-producing strain) and A. flavus (non-ochratoxin-producing strain) were used to amplify a 141 bp fragment of the pks gene. The 141 bp PCR product was successfully cloned into TOPO®TA plasmid. Subsequently, ten-fold dilutions of plasmid DNA were used to generate the standard curve by plotting the threshold cycle against log DNA concentration using qPCR. Further, fungal DNA contamination was quantified in 11 samples of roasted coffee using qPCR. All 11 coffee samples were accepted as safe, since the fungal genomic DNA contamination was less than 3.85 x 10^3 copies. Therefore, this research suggested that qPCR is a fast and accurate method to detect and quantify OTA-producing fungi in coffee products. Thus, we successfully developed a system to quantify fungal contamination in coffee.

Keywords: *Aspergillus carbonarius*, Ochratoxin A, Coffee, Quantitative PCR, Polyketide synthase (*pks*) gene

INTRODUCTION

Ochratoxin A (OTA) is a pentaketide mycotoxin that exhibits immunosuppressive, teratogenic, and carcinogenic properties. OTA is also a potent nephrotoxin and the possible causative agent of Balkan endemic nephropathy in humans (Leong et al., 2006). The toxin can be found in a broad range of processed and unprocessed foodstuffs, including coffee.

Coffee is one of the most popular drinks in the world and a valuable primary product. The presence of OTA in the various stages of coffee processing is of great concern. The major source of OTA in coffee is fungi of the genus *Aspergillus* (Pardo et al., 2004; Velmourougane et al., 2011). Specifically, *Aspergillus carbonarius* is one of the main species responsible for production and accumulation of the toxin in coffee. This fungus has also been reported to have the highest ochratoxigenic potential (Mulè et al., 2006).