Dynamics and Biodiversity of microorganisms (fungi, yeast and bacteria) by PCR-DGGE, influencing OTA production on coffee beans

Durand Noël[1*], El Sheikha Aly[1.2], Fontana Tachon Angélique[1], Montet Didier[1]

[1] UMR Qualisud (CIRAD, Université Montpellier II) 34095 Montpellier Cedex 5, France.

[2] Department of Food Science and Technology (Minufiya University, Faculty of Agriculture), 32511 Shibin El Kom, Egypt.

* Corresponding author: E-mail address: noel.durand@cirad.fr

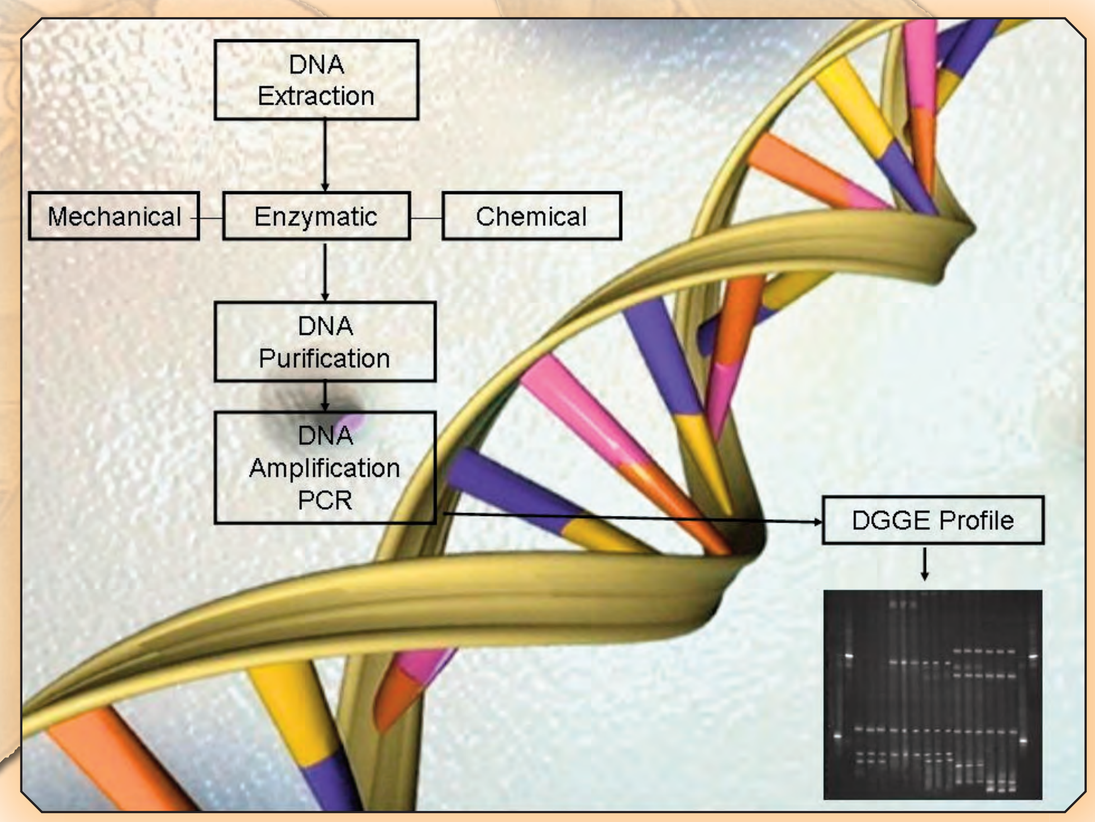
Ochratoxin A is a secondary metabolite produced by various filamentous fungi, is deemed to have nephrotoxic, immunotoxic, teratogenic and carcinogenic effects (1). In tropical zones, OTA is mainly produced in coffee beans by three *Aspergillus* species: *A. carbonarius, A. niger* section Nigri and *A. ochraceus* section Circumdati. In temperate zones *Penicillium verrucosum* and *P. nordicum* are known to synthesize OTA in food commodities (2, 3).

The OTA content in coffee was shown to be closely link to harvesting conditions, post-harvest processing conditions and especially dry processing, storage and transportation conditions (4, 5, 6). In order to understand the OTA contamination process in foodstuffs, assays on PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis) were carried out. PCR-DGGE is a rapid molecular technique that was developed to monitor the dynamics of microbial populations (fungi, yeast and bacteria) per example in coffee mycoflora. In this work, we optimized PCR-DGGE stages: extraction and amplification, repeatability and sensibility of the fungi methodology were tested.

Methodology

• **Sensibility** test was applied to selected Aspergillus species cultivated on Potato Dextrose Agar medium (AES, Combourg, France) for 5 days at 25°C. The fungal spores were collected from plates in aseptic conditions using sterile distilled water with 0.1% Tween 80 solution.
The suspensions of fungal spores were quantified with Thomas cell and dilute in peptone water from 10^7 to 10^2 spores/mL.

• **DNA extraction method,** according to El Sheikha *et al.* (7) different steps of extraction were used (mechanical/enzymatic/chemical). Additionally, the successful application of a eukaryotic universal primer for PCR permitted to amplify and identify many fungi species in one PCR step. The PCR products were analyzed by DGGE by using a Bio-Rad DcodeTM universal mutation detection system (Bio-Rad Laboratories, USA) using the procedure described by El Sheikha *et al.* (8).



Results

• Sensibility

Sensibility was examined on pure cultures of 3 fungal species at different concentrations: *Aspergillus ochraceus, A. carbonarius, A. niger.* Extractions were performed directly on spores. Results were considered as positive when PCR amplification was obtained. The detection limits in pure cultures are shown in Table 1.

Table 1: Detection limits on pure cultures of fungal strains

Fungi/Concentration (spores/mL)	10^7	10^6	10^5	10^4	10^3	10^2
Aspergillus carbonarius	+	+	+	+	-	-
Aspergillus niger	+	+	+	+	-	-
Aspergillus ochraceus	+	+	+	+	-	-

• Repeatability

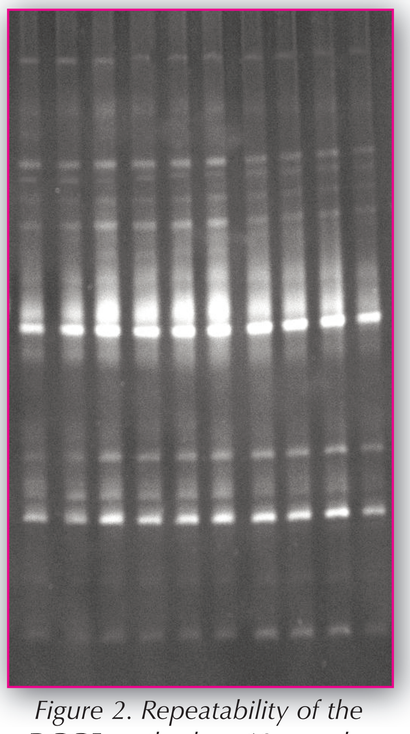


Figure 2. Repeatability of the DGGE method on 10 samples from the same batch.

• Profil DGGE

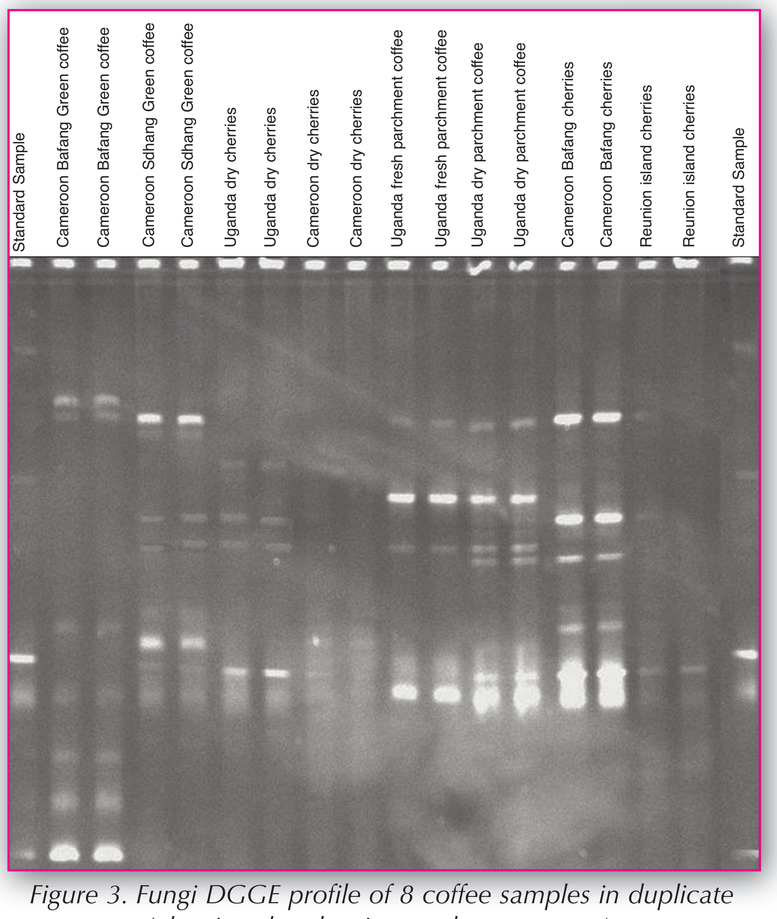


Figure 3. Fungi DGGE profile of 8 coffee samples in duplicate (cherries, dry cherries,parchment, green).

Conclusion

THE sensitivity and the repeatability of the protocol were increased with the improvement of the DNA extraction and the PCR/DGGE conditions (Table 1, Fig. 2).

The dynamics of fungus populations linked to OTA production, as well as post-harvest phytopathogens, could be studied by fingerprinting with PCR-DGGE (Fig. 3). The advantages of this method are its efficiency on all microbial species (fungi, yeast and bacteria) and on the possibility of analysing a wide number of samples (30 samples) in a unique batch. PCR-DGGE provides a method for tracing microorganisms among the technological treatments (Fig. 3).

References

1. IARC (International Agency Research of Cancer), 1993. Some naturally occuring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC monographs on the Evaluation of Carcinogenic Risks to humans. IARC Press, Lyon, 56, 489-521.
2. Pitt J.L., Basilico J.C., Abarca M.L., Lopez C., 2000. Mycotoxins and toxigenic fungi. Medical Mycology 38, 41-46.
3. O'Callaghan, J., Caddick, M.X., Dobson, D.W., 2003. A polyketide synthase gene required for Ochratoxin A biosynthesis in Aspergillus ochraceus. Microbiology-SGM 149, 3485-3491.
4. Paulino de Moreas, M.H., Luchese R.H. Ochratoxin A on Green Coffee: Influence of Harvest and Drying Processing procedures. Journal of Agricultural and Food Chemistry, 2003. 51(19), 5824-5828.
5. Suarez-Quiroz M., Gonzalez-Rios O., Barel M., Guyot B., Schorr-Galindo S., Guiraud J-P. Effect of the post-harvest processing procedure on OTA occurrence in artificially contaminated coffee. International Journal of Food Microbiology. 2005. 103, 339– 345.
6. Bucheli, P., Meyer I., Pittet, A., Vuataz G., Viani R. Industrial storage of Robusta coffee under tropical conditions and its impact on raw material quality and ochratoxin A content. Journal of Agricultural and Food Chemistry, 1998. 46, 4507-4511.
7. El Sheikha A., Condur A., Métayer I., Le Nguyen D. D., Loiseau G., Montet D. (2009a). "Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: An application to Physalis fruits from Egypt". Yeast (in press).
8. El Sheikha A., Le Nguyen D. D., Métayer I., Montet D. (2009b). "An Improved molecular method to analyze the global fungal communities: The PCR-Denaturing Gradient Gel Electrophoresis". Journal of Microbiological Methods (submitted).

References

CIRAD
Centre de coopération internationale en recherche agronomique pour le développement

Design and production: CIRAD, Martine Dupont, August 2009