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Proposal for oral presentation

Dynamics and Biodiversity of microorganisms (fungi and yeast) by PCR-DGGE, with the objective of understanding Ochratoxin production on coffee beans

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Ochratoxin A (OTA) 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 using PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis) were carried. PCR-DGGE is a rapid molecular technique that was developed to monitor the dynamics of microbial populations (fungi, yeast and bacteria) by example in coffee mycoflora. In this work, the PCR-DGGE stages were optimized: extraction and amplification, repeatability and sensibility methodology applied to fungi 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℃. 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).

Conclusion
The dynamics of fungus populations linked to OTA production, as well as post-harvest phytopathogens, could be studied by PCR-DGGE genetic fingerprinting. The advantages of this method are it 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.

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. (2009). "Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: An application to Physalis fruits from Egypt". *Yeast*, 26: 567–573..

8. El Sheikha, A., Métayer, I., Montet, D. (2010). A Biological bar-code for determining the geographical origin of fruit by using 28S rDNA fingerprinting of fungi communities by PCR-DGGE: an application to Physalis fruits from Egypt. *Food Biotechnology*. (**In press**)