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New Strategies for Tracing Foodstuffs: Biological Barcodes Utilising PCR-DGGE

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ABSTRACT

Traceability of foods is undertaken primarily at the administrative level, and the use of advanced analytical tools is rare. Nevertheless, the determination of geographical origin is a demand of the traceability system for the import and export of foodstuffs (UE regulation 178/2002). It is hypothesised that foodstuffs can be traced at source by analysing food sample microbial communities after they have been exported. For this purpose, rDNA profiles generated by PCR-DGGE may be used to detect variability in microbial community (bacteria, yeast, fungi) structures inherent to fish, fruits and grains. This is an emerging traceability tool that imprints food with a unique biological bar code and makes it possible to trace food to its original location. In addition, this analytical technique provides a means to monitor and fully understand the ecology of mycotoxin producing fungi.

KEYWORDS: Traceability; PCR-DGGE; Microbial communities; Foodstuffs; Food safety.

INTRODUCTION

Food traceability is a growing consumer concern worldwide. In view of the difficulties involved in installing documentary systems in developing countries and in following foodstuffs through the production process, one possible approach is to identify and validate molecular fingerprinting based on the food's environment to assure traceability. Currently, there are no analytical methods available that permit the efficient determination of foodstuff origin or that allow them to be followed during international trade. In case of doubt or fraud, it is necessary to find a precise and fast analytical technique to assign geographical origin.[1]

The most popular analytical methods used to ensure the determination of origin are bar codes and stable isotopes.[2] Stable isotopes are currently used for reference by the EU to determine to origin of wine.[3] It thus seems difficult to use fruit genomic markers to ensure the traceability of Shea tree fruits. However, the skin of fresh fruits is not sterile and can carry microorganisms or their fragments. The presence of various microorganisms depend on the external environment of the fruit (soil ecology, spoilage, insects, diseases), but microorganisms also result from human activity.[4] The use of molecular biological methods in general or by PCR-DGGE in particular have been described.[5] These tools may be used to deliver reliable results in an efficient and acceptable manner to determine the origin of food products.

WHY DOES THE NEED ARISE TO USE MOLECULAR TECHNIQUES TO TRACE FOODSTUFFS?

In past years, the development of biological identification technologies has contributed to the industry's ability to support and validate traceability systems. In parallel, computer technology has provided the industry with many new and innovative tools with which to trace

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products.[6] Biological, analytical and informatics tools have been synergistically proposed and utilised for traceability in the wine industry.[7] Currently, there are no molecular biological techniques available to determine the geographical origin of food. The idea was to create a "biological barcode"[8] based on the analysis of the DNA of microorganisms present on the products. This method is based on the assumption that the microbial communities found on foodstuffs are specific to a geographical area.[5,9]

LINKAGE BETWEEN TRACEABILITY AND FOOD SAFETY

Recently, tracking and tracing systems have become the most important methods used to ensure food safety, whereas food safety is an intrinsic part of food quality. A reliable traceability system means that a tool can allow a food company to track and trace any foodstuff which does not meet consumer expectations or the applicable regulations in an importing country. The main objective of a traceability system is to tell a product's story, i.e., identify a unique product batch and the raw materials used in its production and follow that batch through its production and distribution all the way to the retailer. Today, tracking and traceability software tools are of major interest to the retail business (as a business to business communication tool). Tracking and traceability systems can be incorporated into information systems where consumers can receive information on any product. Traceability systems enable efficient product recall and allow fewer products to be recalled. This can bring important cost savings, where the aim is to provide consumers with nutritious and healthy products which are produced in a cost-efficient way.[10,11]

WHY PCR-DGGE?

The Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) is a well-established molecular tool in environmental microbiology which allows for the study of complexity and behaviour of microbial ecology. The PCR-DGGE is capable of providing a fingerprint of the microbial community in a food sample after direct DNA extraction (Figure 1). Briefly, a food sample is subjected to DNA extrac-

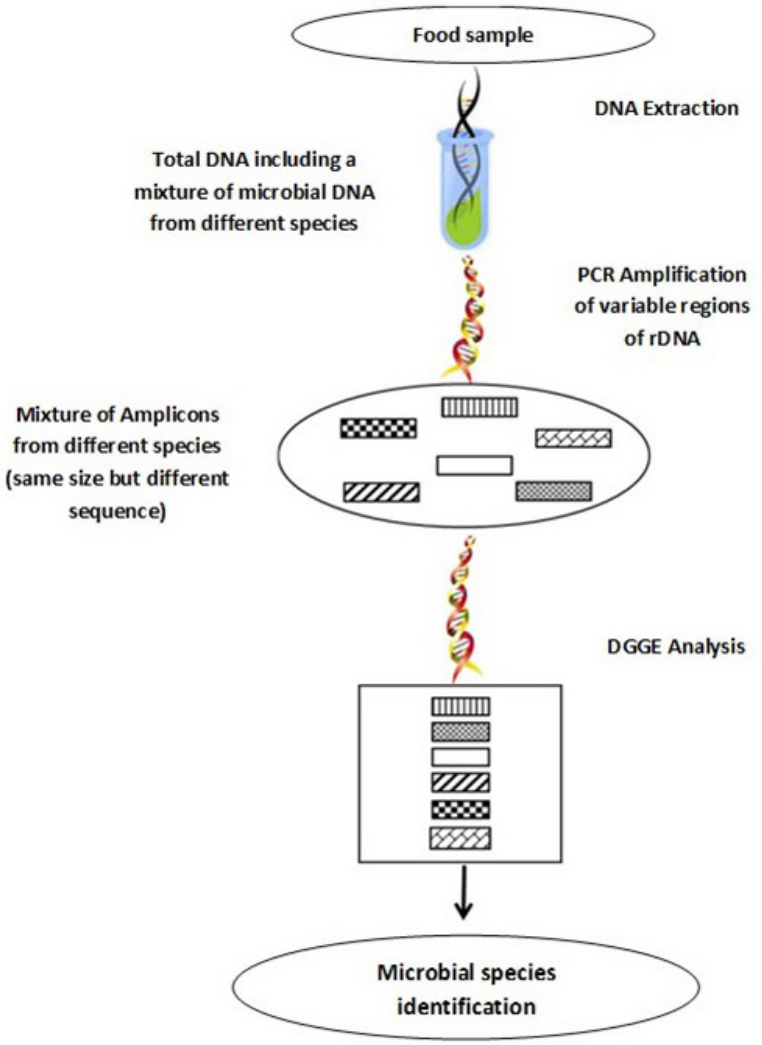

Figure 1: How PCR-DGGE works step by step with food samples.[5]

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tion, with the attainment of a mixture containing DNA from the microbial species that is present in the sample. Successively, the DNA mixture is used as a template for PCR amplifications of specific variable DNA regions of taxonomic interest by obtaining an amplified product that is a mixture of amplicons from the species present in the initial sample. All amplicons are of the same size but with different sequences, and can be thus separated by DGGE. The final result is a fingerprint that is specific to the analysed sample and contains a series of bands relative to the microbial species that is present. Identification of the species can be achieved by purifying and sequencing the bands in the DGGE profile.[1,12] The most commonly employed target for PCR amplification prior to DGGE is the ribosomal DNA. This is because ribosomal DNA is considered the most conserved gene in all cells inclusive of variable regions.[13] The technique is reliable, reproducible, rapid, inexpensive and capable of analysing a large number of samples in a single step. DGGE is applied to the study of microbial diversity and can be coupled with techniques of cloning and subsequent sequencing.[14] The PCR-DGGE has the advantage that separation does not depend on the size of the fragment, but rather on the melting behaviour of the PCR product. DGGE is more discriminating than is Restriction Fragment Length Polymorphism (RFLP).[15] In addition, the banding pattern obtained from the PCR products is indicative of different species,[16] or species assemblages,[14] and allows visualisation of the genetic diversity of microbial population indices to quantify biodiversity,[17,18] and it has the potential to find new noncultural microorganisms.[19] One of the characteristics of strong DGGE is the ability to identify community members by sequencing and by re-amplifying bands excised directly from gels or by hybridisation analysis with specific probes,[18,20] which is not possible with RFLP.[18,21] Despite these limitations, DGGE is strongly preferred and is considered one of the best techniques for monitoring the microbial community of foodstuffs in a comprehensive, rapid and reproducible manner.[5,9,22-25]

APPLICATIONS OF PCR-DGGE TECHNIQUE IN THE TRACEABILITY OF FOODSTUFFS

For this purpose, molecular techniques employing rDNA profiles generated by PCR-DGGE were used to detect the variation in microbial community (bacteria, yeast, fungi) structures of fish,[11,26-29] fruits,[1,12,30-34] salt,[35] cheeses,[36] grains,[37-39] and organic and conventional foods.[40] These studies demonstrated that microbial communities were specific for each location, allowing for the foodstuffs to be differentiated. Several microbial species were identified as potential biological markers, whose detection could be used to certify the origin as well as the mode of production of the foodstuff.

PCR-DGGE Technique in the Traceability of Fish

Analysis of bacterial communities in fish samples has often been investigated using culture dependent methods and culture-independent methods by Random Amplified Polymorphic DNA (RAPD).[41] Aquatic microorganisms are known to be closely associated with the physiological status of fish.[26,41-43] The water composition, temperature and weather conditions can influence the bacterial communities.[44,45] The predominant microbial flora (i.e., bacteria, yeast) would permit the determination of the capture area, production process or hygienic conditions during post-harvest operations, yet there are very few published works that provide an analysis of the microbial communities in fish samples by PCR-DGGE and differentiate geographical location.[26-29]

PCR-DGGE Technique in the Traceability of Fruits

Fruits are included in the priority list of many governments' horticulture and fruit export plans.[34] For economic reasons and for profitability, batches of fruits representing various species or various cultures could be mixed. It is thus very difficult to check their exact geographical origin. Traceability is only assured by rigorous labelling and administrative documentation without proper analytical control. In case of doubt or fraud, it is necessary to find a precise and rapid analytical technique to determine their geographical origin.[31] The PCR-DGGE method of analysis is a unique way to generically identify all microbial flora (bacteria, yeast, moulds) present on fruit, in order to create the linkage of microbial communities to the geographical origin and avoid the individual analysis of each strain. The acquired band patterns for the microbial communities of different species of fruits and different harvesting locations were compared and analysed statistically to determine the fruit geographical origin.[30-33] Figure 2 shows the DGGE pattern of the DNA of yeast communities of Shea tree fruits from two different regions of Mali, while figure 3 illustrates the cluster analysis of the DGGE gel patterns, which explains that the DGGE pattern of the DNA of yeast communities of Shea tree fruits was strongly linked to the microbial environment of the fruit.[46]

PCR-DGGE Technique in the Traceability of Grains

As an example, coffee could be attacked by pathogenic microorganisms (including mycotoxigenic fungi) which could have a serious impact on coffee quality.[47] The presence of various microflora depends on the external environment of the coffee (soil ecology, spoilage, insects, diseases), but also on microbial communities brought about by human activity.[4] Undesirable microorganisms present on coffee beans before and/or during transformation can cause detrimental, sensorial or chemical defects. Fungi are responsible for coffee diseases (mildew and black rot),[48] mycotoxin production,[49-51] or sensorial defects in coffee such as musty or earthy aromas.[52] Knowledge of the structure and diversity of the fungal communities of coffee beans would lead to a better understanding of the emergence of defects in coffee in relation to the fungi present on coffee beans.[37,38]

PCR-DGGE has proven to be a rapid and effective method that can be used to describe fungal communities on coffee beans.[53] This confirms the idea put forward by Laforgue et al.[54] who showed that PCR-DGGE was an effective and quick

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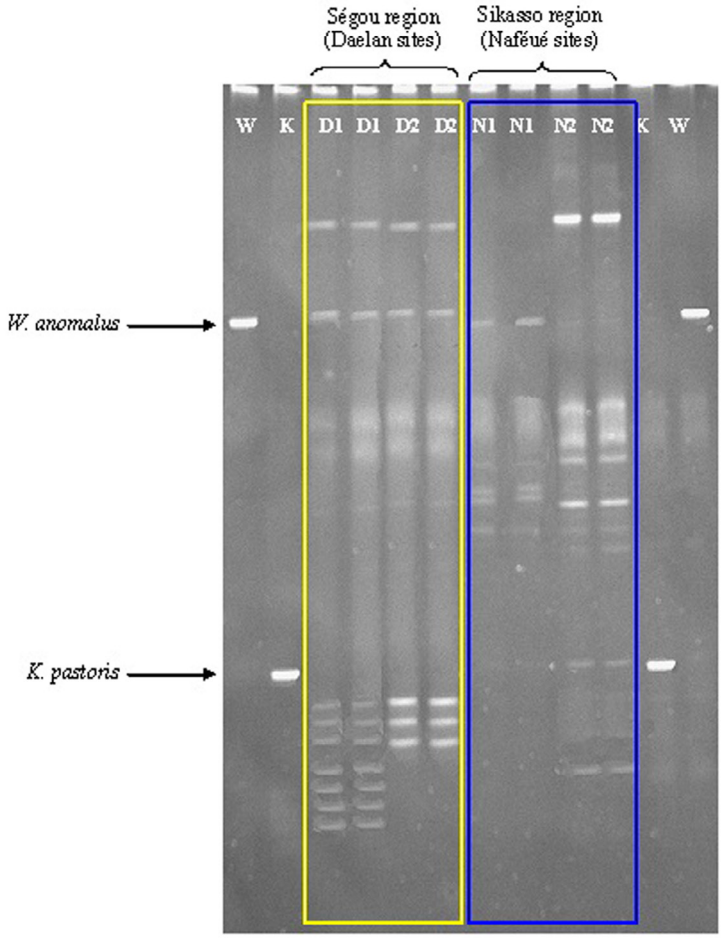

Figure 2: DGGE Profiles of 26S rDNA for yeast strains isolated from Shea tree fruits from two different regions of Mali: Ségou region (D1, D2: Daelan sites) and Sikasso region (N1, N2: Naféguésites).[46]

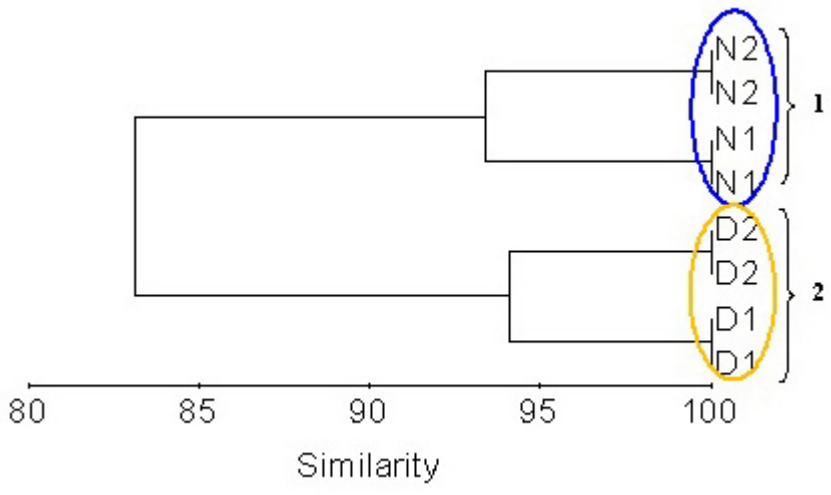

Figure 3: Cluster analysis of 26S rDNA profiles for yeast strains isolated from Shea tree fruits from two different regions of Mali: Ségou region (D1, D2: Daelan sites) and Sikasso region (N1, N2: Naféguésites).[46]

method to follow food product fungal communities. Nganou et al.[37] used PCR-DGGE to permit the certification of coffee origin by using 28S rDNA fingerprinting of moulds.

CONCLUSIONS: CHALLENGES AND PROSPECTS

Universal scientific methods for the determination of geographical origin of a foodstuff do not, in fact, exist. There are only indirect methods available, which often must be coupled together to increase their accuracy. Methods which permit the analysis of the micro environment of food are very promising and must be better studied by research teams around the world.[11]

PCR-DGGE is strongly preferred and considered one of the best techniques for monitoring the microbial communities associated with food samples in a comprehensive, rapid and reproducible manner. Also, by PCR-DGGE, it is demonstrated that there is a link between the microbial populations and the geographical area of origin of the foodstuff. So, this method is proposed to be an analytical traceability tool for foodstuffs.[9]

The main problem will be the construction of the data banks that are necessary for the PCR-DGGE technique. Other techniques will be developed in the near future, taking into account, for example, the micro-constitution of food. One could consider the micro-components of lipids like to copherols, phospholipids, sterols or other molecules brought into the environment, like pesticides, traces of insects, heavy metals, radioactive isotopes, et al.[11]

REFERENCES

1. El Sheikha AF, Bouvet J-M, Montet D. Biological bar-code for the determination of geographical origin of fruits by using 28S rDNA fingerprinting of fungal communities by PCR-DGGE: An application to Shea tree fruits. *Quality Assurance and Safety of Crops & Foods*. 2011; 3(1): 40-47. doi: 10.1111/j.1757-837X.2010.00090.x

2. Peres B, Barlet N, Loiseau G, Montet D. Review of the current methods of analytical traceability allowing determination of the origin of foodstuffs. *Food Control*. 2007; 18: 228-235. doi: 10.1016/j.foodcont.2005.09.018

3. Ghidini S, Ianieri A, Zanardi E, et al. Stable isotopes determination in food authentication: a review. *Annals of the Faculty of Veterinary Medicine, University of Parma University.* 2006; XXVI: 193-204.

4. Sodeko OO, Izuagbe YS, Ukhun ME. Effect of different preservative treatment on the microbial population of Nigerian orange juice. *Microbios*. 1987; 51: 133-143.

5. El Sheikha AF. Determination of geographical origin of Shea tree and Physalis fruits by using the genetic fingerprints of the microbial community by PCR/DGGE. Analysis of biological properties of some fruit extracts. PhD Thesis. Montpellier University II, Mont-pellier, France; 2010.

6. Raspor P. Bio-markers: traceability in food safety issues. *Acta Biochimica Polonica*. 2005; 52: 659-664.

7. Pinder R, Meredith C. Wine-A Scientific Exploration. UK: Taylor and Francis; 2003.

8. Montet D, Leesing R, Gemrot F, Loiseau G. Development of an efficient method for bacterial diversity analysis: Denaturing Gradient Gel Electrophoresis (DGGE). In: Seminar on Food Safety and International Trade. Bangkok, Thailand; 2004.

9. El Sheikha AF. Determining the Geographical Origin Fruit: Examples Shea and Physalis by Using Genetic Footprints on the Community Micro-Bienne PCR / DGGE. Saarbrücken, Germany: European Academic Publishing, GmbH& Co. KG; 2011.

10. AquaTT. Traceability in Aquaculture. Website: http://www.piscestt.com/. 2004; Accessed 2015.

11. El Sheikha AF, Montet D. How to determine the geographical origin of seafood? Critical *Reviews in Food Science and Nutrition*. 2015. doi: 10.1080/10408398.2012.745478

12. El Sheikha AF, Durand N, Sarter S, Okullo JBL, Montet D. Study of the microbial discrimination of fruits by PCR-DGGE: application to the determination of the geographical origin of Physalis fruits from Colombia, Egypt, Uganda and Madagascar. *Food Control*. 2012; 24(1-2): 57-63. doi: 10.1016/j.foodcont.2011.09.003

13. Smit S, Widmann J, Knight R. Evolutionary rates vary among rRNA structural elements. *Nucleic Acids Research.* 2007; 35: 3339-3354. doi: 10.1093/nar/gkm101

14. Muyzer G, De Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*. 1993; 59: 695-700.

15. Moeseneder MM, Arrieta JM, Muyzer G, Winter C, Herndl GJ. Optimization of terminal restriction fragment length polymorphism analysis for complex marine bacteriop-lankton communities and comparison with denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*. 1999; 65(8): 3518-3525.

16. Vaughan EE, Mollet B, deVos WM. Functionality of probiotics and intestinal lactobacilli: light in the intestinal tract tunnel. *Current Opinion in Biotechnology.* 1999; 10(5): 505-510. doi: 10.1016/S0958-1669(99)00018-X

17. Simpson JM, McCracken VJ, White BA, Gaskins HR, Mackie RI. Application of denaturant gradient gel electrophoresis for the analysis of the porcine gastrointestinal microbiota. *Journal of Microbiological Methods*. 1999; 36(3):167-179. doi: 10.1016/S0167-7012(99)00029-9

18. McCracken VJ, Simpson JM, Mackie RI, Gaskins HR. Molecular ecological analysis of dietary and antibiotic-induced alterations of the mouse intestinal microbiota. *Journal of Nutrition*. 2001; 131(6): 1862-1870.

19. Simpson JM, McCracken VJ, Gaskins HR, Mackie RI. Denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA amplicons to monitor changes in fecal bacterial populations of weaning pigs after introduction of Lactobacillusreuterii strain MM53. *Applied and Environmental Microbiology*. 2000; 66(11): 4705-4714. doi: 10.1128/AEM.66.11.4705-4714.2000

20. Temmerman R, Scheirlinck I, Huys G, Swings J. Culture-independent analysis of probiotic products by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*. 2003; 69(1): 220-226. doi: 10.1128/AEM.69.1.220-226.2003

21. Muyzer G. DGGE/TGGE: a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology*. 1999; 2(3): 317-322. doi: 10.1016/S1369-5274(99)80055-1

22. Ercolini D. PCR-DGGE fingerprinting: Novel strategies for detection of microbes in food. *Journal of Microbiological Methods*. 2004; 56(3): 297-314. doi: 10.1016/j.mimet.2003.11.006

23. Montet D, El Sheikha AF, Le Nguyen DD, Condur A, Métayer I, Loiseau G. Determine the origin of food through molecular biology. The example of PCRDGGE. *Biofutur*. 2010; 307: 36-38.

24. El Sheikha AF, Montet D. The Determination of geographical origin of foodstuffs by using innovative biological barcode. *Journal of Life Sciences*. 2012; 6(12): 1334-1342. doi: 10.17265/1934-7391/2012.12.004

25. Vaz-Moreira I, Egas C, Nunes OC, Manaia CM. Bacterial diversity from the source to the tap: a comparative study based on 16S rRNA gene-DGGE and culture-dependent methods. *FEMS Microbiology Ecology*. 2013; 83(2): 361-374. doi: 10.1111/1574-6941.12002

26. Leesing R. Identification and validation of specific markers for the traceability of aquaculture poisons in their import / export, p. 183. PhD Thesis, Montpellie University 2, France; 2005.

27. Le Nguyen DD, Hanh HN, Dijoux D, Loiseau G, Montet D. Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: an application on Pangasius fish from Viet Nam. *Food Control*. 2008; 19(5): 454-460. doi: 10.1016/j.foodcont.2007.05.006

28. Tatsadjieu NL, MaïworéJ, Hadjia MB, Loiseau G, Montet D, Mbofung CMF. Study of the microbial diversity of Oreochromisniloticus of three lakes of Cameroon by PCR-DGGE: Application to the determination of the geographical origin. *Food Control*. 2010; 21(5): 673-678. doi: 10.1016/j.foodcont.2009.10.006

29. Montet D, Le Nguyen DD, Kouakou AC. Determination of fish origin by using 16S rDNA fingerprinting of microbial communities by PCR-DGGE: An application on fish from different tropical origins. In: Muchlisin Z, ed. Aquaculture. Rijeka, Croatia: InTech; 2012: 93-108.

30. Le Nguyen DD, Gemrot E, Loiseau G, Montet D. Determination of citrus fruit origin by using 16S rDNA fingerprinting of bacterial communities by PCR- DGGE: an application on clementine from Morocco and Spain. *Fruits*. 2008; 63: 3-9. doi: 10.1051/fruits:2007049

31. El Sheikha AF, Condur A, Métayer I, Le Nguyen DD, Loiseau G, Montet D. Determination of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: preliminary application to Physalis fruits from Egypt. *Yeast*. 2009; 26(10): 567-573. doi: 10.1002/yea.1707

32. El Sheikha AF. Determination of the geographical origin of fruits by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: An application to Shea tree fruits. *Journal of Life Sciences*. 2010; 4(6): 9-15. doi: 10.17265/1934-7391-/2010.06.002

33. El Sheikha AF, Montet D. Determination of fruit origin by using 28S rDNA finger-printing of fungi communities by PCR-DGGE: An application to Physalis fruits from Egypt, Uganda and Colombia. *Fruits*. 2011; 66(2): 79-89. doi: 10.1051/fruits/2011001

34. El Sheikha AF, MétayerI, Montet D. 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*. 2011; 25(2): 115-129. doi: 10.1080/08905436.2011.576556

35. DufosséL, Donadio C, Valla A, Meile J-C, Montet D. Determination of speciality food salt origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: An application on marine salts produced in solar salterns from the French Atlantic Ocean. *Food Control*. 2013; 32(2): 644-649. doi: 10.1016/j.foodcont.20s13.01.045

36. Arcuri EF, El Sheikha AF, Rychlik T, Métayer I, Montet D. Determination of cheese origin by using 16S rDNA fingerprinting of bacteria communities by PCReDGGE: Preliminary application to traditional Minas cheese. *Food Control*. 2013; 30(1): 1-6. doi: 10.1016/j.foodcont.2012.07.007

37. Nganou DN, Durand N, Tatsadjieu NL, et al. Determination of coffee origin by using 28S rDNA fingerprinting of fungal communities by PCR-DGGE: application to the Ca-meroonian coffee. *International Journal of Bioscience*. 2012; 2(5): 18-30.

38. Durand N, El Sheikha AF, Suarez-Quiros M-L, et al. Application of PCR-DGGE to the study of dynamics and biodiversity of yeasts and potentially OTA producing fungi during coffee processing. *Food Control*. 2013; 34(2): 466-471. doi: 10.1016/j.foodcont.2013.05.017

39. Hamdouche Y, Guehi T, Durand N, Kedjebo KBD, Montet D, Meile J-C. Dynamics of microbial ecology during cocoa fermentation and drying: Towards the identification of molecular markers. *Food Control*. 2015; 48: 117-122. doi: 10.1016/j.foodcont.2014.05.031

40. Bigot C, Meile J-C, Kapitan A, Montet D. Discriminating organic and conventional foods by analysis of their microbial ecology: An application on fruits. *Food Control*. 2015; 48: 123-129. doi: 10.1016/j.foodcont.2014.03.035

41. Spanggaard B, Huber I, Nielsen TJ, Nielsen T, Appel K, Gram L. The microbiota of rainbow trout intestine: a comparison of traditional and molecular identification. *Aquaculture*. 2000; 182: 1-15.doi: 10.1016/S0044-8486(99)00250-1

42. Grisez L, Reyniers J, Verdonck L, Swings J, Ollevier F. Dominant intestinal microbiota of sea bream and sea bass larvae, from two hatcheries, during larval development. *Aquaculture*. 1997; 155: 387-399. doi: 10.1016/S0044-8486(97)00113-0

43. Al Harbi AH, Uddin N. Quantitative and qualitative studies on bacterial flora of hybrid tilapia (Oreochromisniloticusx O. aureus) cultured in earthen pond in Saudi Arabia. *Aquaculture*. 2003; 34: 43-48. doi: 10.1046/j.1365-2109.2003.00791.x

44. Wong HC, Chen MC, Liu SH, Liu DP. Incidence of highly genetically diversified Vibrioparachaemolyticus in seafood imported from Asian countries. *International Journal of Food Microbiology*. 1999; 52: 181-188.doi: 10.1016/S0168-1605-(99)00143-9

45. De Sousa JA, Silva-Sousa AT. Bacterial community associated with fish and water from Congohas River, Sertaneja, Parana, Brasil. *Brazilian Archives of Biology and Technology*. 2001; 44: 373-381. doi: 10.1590/S1516-89132001000400007

46. El Sheikha AF, Bouvet J-M, Montet D. Novel molecular fingerprinting for the geographical origin of fruits. *Mansoura Journal of Biology*. 2011; 37(2): 35-43.

47. Silva CF, Schwan RF, Dias ES, Wheals AE. Microbial diversity during maturation and natural processing of coffee cherries of Coffeaarabicain Brazil. *International Journal of Food Microbiology*. 2000; 60: 251-260. doi: 10.1016/S0168-1605-(00)00315-9

48. Bieysse D, Manga B, Bedimo M, et al. The coffee berry a potential threat to the global Arabica culture. In: Research and coffee growing. Montpellier, France: CIRAD-CP; 2002: 145-152.

49. Cabañes FJ, Accensi F, Bragulat MR, et al. What is the source of ochratoxin A in wine? *International Journal of Food Microbiology*. 2002; 79(3): 213-215. doi: 10.1016/S0168-1605-(02)00087-9

50. Battilani P, Pietri A, Bertuzzi T, Languasco L, Giorni P, Kozakiewicz Z. Occurrence of ochratoxin A-producing fungi in grapes grown in Italy. *Journal of Food Protection*. 2003; 66(4): 633-636.

51. Fungaro MHP, Sartori D. Genetic relationships among strains of the Aspergillusniger aggregate. *Brazilian Archives of Biology and Technology*. 2009; 52: 1-9. doi: 10.1590/S1516-89132009000700031

52. La Guerche S, Garcia C, Darriet P, Dubourdieu D, Labarère J. Characterization of Penicillium species isolated from grape berries by their Internal Transcribed Spacer1 (ITS1) sequences and by gas chromatography-mass spectrometry analysis of geosmin production. *Current Microbiology*. 2004; 48(6): 405-411. doi: 10.1007/s00284-003-4176-4

53. Masoud W, Cesar LB, Jespersen L, Jakobsen M. Yeast involved in the fermentation of Coffeaarabica in East Africa determined by genotyping and by direct denaturing gradient gel electrophoresis. *Yeast*. 2004; 21(7): 549-556. doi: 10.1002/yea.1124

54. Laforgue R, GuérinL, Pernelle JJ, Monnet C, Dupont J, Bouix M. Evaluation of PCR-DGGE methodology to monitor fungal communities on grapes. *Journal of Applied Microbiology*. 2009; 107(4):1208-1218. doi: 10.1111/j.1365-2672.2009.04309.x