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USE OF FUNGAL COMMUNITIES FINGERPRINTING TO DISCRIMINATE FARMING TYPE BY USING 28 S rDNA ANALYZED BY PCR-DGGE: AN APPLICATION TO PEACH FRUITS

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ABSTRACT

Detection of farming type became an important issue for the traceability system of both import and export foodstuff. First tracing hypothesis for the source of a product is via global analysis for the food microbial communities and by trying to link the food statistically to the farming type. The variation in fungal communities' structures of peach fruits was detected by employing 28S rDNA profiles generated by PCR-DGGE. We applied this novel technology on Jordan peaches from two different farming modes, which were organic and treated fruits from conventional farming. The Factorial Correspondence Analysis (FCA) for the 28S rDNA profiles revealed the presence of distinct fungal communities for each farming type. This technique is a novel traceability tool which supply food with a unique biological bar code and permit to trace back the food to their farming modes.

Keywords: *peach ,traceability, PCR-DGGE, fungal communities, farming modes.*

INTRODUCTION

Traceability is considered nowadays in relation to food like “buzzword”, especially after a series of food safety incidents. Consumers become more and more curious and very sensitive to foodstuffs quality and farming type that they buy.

Traceability can be defined as the ability to know the full history, origin or use of an article or its contents or an activity through a registered method (ISO, 2007). With regard to the difficulties of installing the documentary systems in developing countries, and to follow up the processing of a product, we can make a proposal to identify and validate some linked biological markers that comes the food farming type in order to make their traceability assurance, There are only a few current analytical tools nowadays which provide the food farming type efficient detection. But in the case of doubtful, it is very important to find a fast accurate analytical tool that will enable us to detect their farming modes.

Peach (*Prunus persica*) belongs to the Rosaceae family (i.e. the rose family) which is a medium sized group of flowering plants, that include many genera and species. This family

name is derived from the genus Rosa. There are several economic valuable products that come from this family, that include many edible fruits like peaches, apples, apricots, etc., in addition to some ornamental shrubs and trees.

Denaturing Gradient Gel Electrophoresis (DGGE) is a genetic global fingerprinting technique used to make separation of multiple DNA sequences based on their mobility in increasingly denaturing conditions. DGGE is based up on melting behavior differences of small DNA fragments (200-700 bp); even a single base substitution can cause such a difference (Mahdieh and Rabbani, 2013).

The main motive target of this paper work was to create a "biological profile" (Montet *et al.,* 2004) based up on Jordan peaches fungal DNA analysis. This technique is based on assumption that the fruits fungal populations are specific and unique for each farming mode (Le Nguyen *et al.,* 2008; Montet *et al.,* 2008; El Sheikha *et al.,* 2009).

MATERIALS AND METHODS

Sampling of fruits :

Mature peaches were collected from two different farming modes in Jordan, which are organic (control fruits) and treated fruits from conventional farming. Fruits were directly collected from the tree using gloves and kept in sterile bags (in order to preserve their initial micro flora) on beginning of Oct. 2012. The collected samples were stored in a refrigerator then transferred by plane to Cirad - Montpellier (France).

Fungal DNA Extraction from Jordan peach fruits :

This technique was carried out according to the protocol described by El Sheikha *et al.,* (2011).

PCR- DGGE analysis :

Part of the region of the 28S rDNA gene was amplified using eukaryotic universal primers U1 (50-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GTG AAA TTGTTG AAA GGG AA-30, Sigma company) and the reverse primer U2 (50-GAC TCC TTG GTC CGT GTT-30, Sigma company) amplifying about 260 bp fragment (Wu *et al.,* 2002; Li *et al.,* 2008). A 35 bp GC-clamp (Sigma Company) was added to the forward primer in order to ensure that DNA fragment will remain partially double stranded and also screened region is in the lowest melting domain (Sheffield *et al.,* 1989).

PCR was carried out in a final volume (50 mL) including 2.5 mL DMSO, 0.4 mM of each primers, deoxyribonucleotide triphosphate, 200 mM, 3mM $MgCl_2$, and 5mL of 10 x of reaction Taq buffer Mg Cl_2 free, 1.25U of Taq DNA polymerase and 2 mL of the fungal extracted DNA. The amplification was performed as following: initial denaturation at 94°C for 3 min, 30 cycles of 94°C for 45 sec, 50 °C for 50 sec and 72°C for 90 sec, and a final extension at 72°C for 5 min.

About 5 µL of PCR products were analyzed initially via conventional electrophoresis in 2% (w/v) agarose gel with TAE 1x buffer, then subjected to ethidium bromide staining (50 $mg.mL^{-1}$ in TAE 1x) and, quantified via standard (DNA mass ladder 100 bp,

Promega company).

The resulted PCR products were analyzed by DGGE using a Bio- Rad DcodeTM universal mutation detection system (Bio- Rad Laboratories, Benicia, CA, USA).

Samples containing nearly equal amounts of PCR amplicons were loaded into 8% (w/v) polyacrylamide gels (acrylamide/ N, N'methylene bisacrylamide, 37.5/1, Promega Company) in 1x TAE buffer (40 mM Tris-HCl pH 7.4, 20 mM sodium acetate, 1 mM Na_2–EDTA).

All of the electrophoresis runs were carried out at 60ºC via denaturing gradient ranging from (30–40%) to (60–70%), and were finally standardized at 40–70% (100% corresponded to 7 M urea and 40% (v/v) formamide).

The gels were electrophoresed at 20 volts for 10 min and then at 80 volts for 16 h. After electrophoresis, gels were stained via ethidium bromide for 30 min and rinsed in distilled water for 20 min and then photographed via a UV transilluminator with the Gel Smart 7.3 system (Clara Vision).

Statistical and Image analysis :

The gel photos individual lanes were straightened and aligned via Image - Quant TL - software, v.2003. This software allowed identifying the relative positions of bands.

The resulted banding pattern in the DGGE analysis represents a complete photo of all of the dominant fungi (molds) in the populations. Any individual distinct band refers to a unique "sequence type" (Muyzer *et al.,* 1995). This was ensured by Kowalchuk *et al.* (1997), who showed that co-migrating bands generally linked to identical sequence. The PCR-DGGE fingerprints were scored manually via the presence and absence of co - migrating bands, regardless of their intensity. Pair wise community similarities were quantified via the Dice similarity coefficient (SD) (Heyndrickx *et al.,* 1996).

"SD = 2 Nc / Na + Nb "

Where, Na refers to the bands number recorded in A sample, Nb refers to the bands number in B sample, and Nc refers to the bands numbers that are common into both samples. The Similarity index was expressed ranging from 0 (complete dissimilarity) to 100 (perfect similarity). Valuable differences of fungal communities of peach fruits from two farming types were determined via the Factorial Correspondence Analysis (FCA) using the first two variances which described most of the variation in whole data set.

RESULTS

Fungal DNA Extraction from Jordan peach fruits :

The DNA of the fungal communities was extracted on Jordan peach fruits and the extraction was verified on a 0.8% (w/v) agarose gel.

Confirmation of the PCR amplification for the extracted fungal DNA:

The obtained fungal DNA extracted from Jordan peach fruits was amplified via normal PCR procedures. The PCR products (amplicons) were electrophoresed on 2% (w/v) agarose gel at 100 V for 30 min in the TEA buffer. All of the obtained bands were clearly noted at a molecular size of 260 bp, which was the ex-

pected molecular size for these amplicons (Figure 1). The band intensities (PCR amplicons) were very important proving the success of PCR reaction, which allowed us to continue these amplicons analysis by DGGE method.

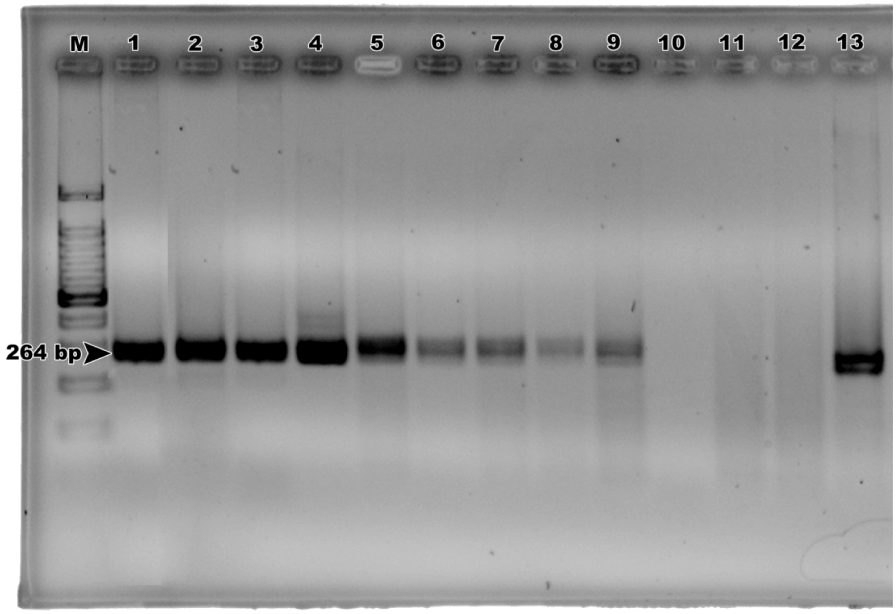

Fig. (1) : Fungal communities PCR Amplicons using Gc U1 f & U2r primers (264 bp). Lanes 1-4 are c2,c3,c4,c5 for organic farming while lanes 5-9 are f1-5 for the conventional farming.
Lane 13: +ve control Aspergillus niger Lane12: –ve control

Comparison of DGGE pattern of Jordan peach fruits fungal DNA extracted from different farming types :

Regarding to the DGGE gel, the obtained bands had enough intensities that enable us to analyze and discriminate the fungal DNA extracted from Jordan peach fruits via different farming modes (Figure 2). So the total DNA quantity that were deposited in the DGGE gel wells were enough to use fungal DNA as potential biological markers to discriminate these two farming modes.

Each of the vertical lines belonged to a fruit farming mode and each of the spots referred to fungal species. The fungal PCR-DGGE replica patterns of Jordan peaches for each farming mode were similar and showed the presence of 8–14 bands for each peach fruit.

Also, we noted that there were two different sets of bands (spots) in the gel; first set consisted of 6 bands that were unique for the organic farming mode and the second set of only 2 bands that was also unique for the conventional farming mode. So, the two farming modes were clearly discriminated via 28 S rDNA analysis of Jordan peaches.

Also, by the way, FCA proved to be a potent statistical tool to discriminate the fungal communities of Jordan peach samples from the two different farming modes (Figure 3), as

we can observe clearly that the two different farming types were represented by two different groups via this statistical analysis facility.

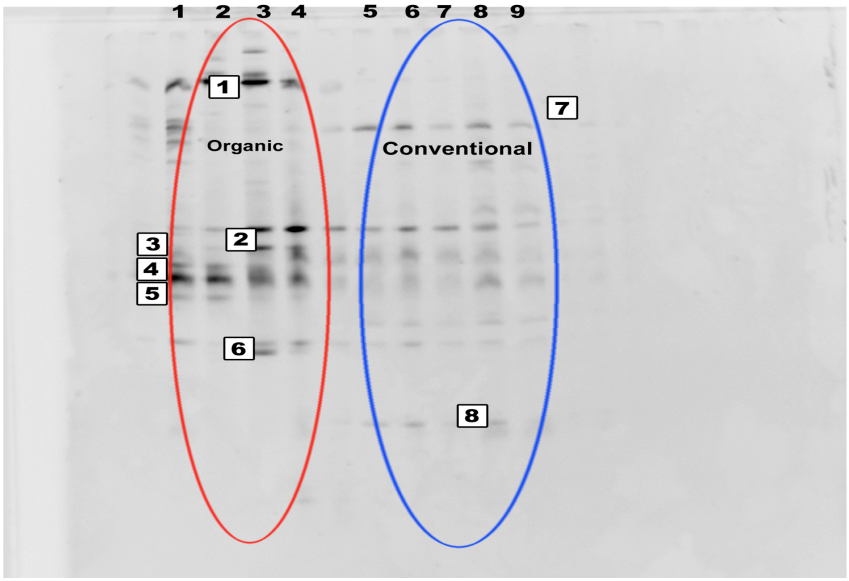

Fig. (2) : PCR-DGGE profiles of 28S rDNA of Jordan peach fruits from two different farming types (organic and conventional)
Lanes 1-4 are c2, c3, c4, c5 for organic farming type while lanes 5-9 are f1-5 for Conventional farming.

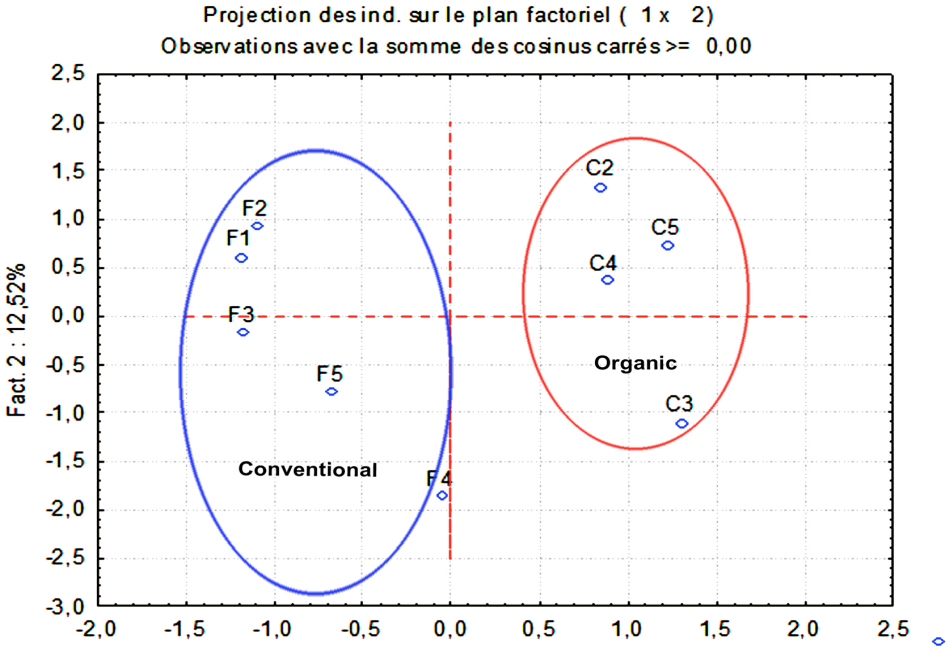

Fig. (3) : PCR-DGGE profiles of 28S rDNA of Jordan peach fruits from two different farming types (organic and conventional)
Lanes 1-4 are c2, c3, c4, c5 for organic farming type while lanes 5-9 are f1-5 for Conventional farming.

DISCUSSION

Some researchers already used PCR – DGGE technique to analyze the fungal communities in fruits (Fleet, 2007; Prakitchaiwattanaa *et al.,* 2007); but we think that this paper is one of the few papers that used the fungal communities analysis of Jordan peach fruits analyzed by the global molecular tool PCR-DGGE. We confirmed that the DGGE banding pattern of the Jordan peach fruits fungal 28 S rDNA communities was strongly related to the farming mode of the fruits. The differences in the band profiles could be due to different farming modes and the types of treatment applied could also affect the peach fungal communities.

PCR-DGGE has many applications. PCR-DGGE technique has been already used to investigate and study several patterns of microorganisms such as yeasts and bacteria (Muyzer *et al.,* 1998; El-Latif *et al.,* 2006; Wu *et al.,* 2006, Montet *et al.* 2008). El-Latif *et al.,* (2006) pointed out that five yeast strains could be differentiated from each other in the DGGE profile.

The FCA statistical analysis of DGGE pattern revealed that there was a complete statistical correspondence between the farming mode and mold communities. Where upon we could create a statistical link between the fungal populations and the farming modes.

CONCLUSION

Mature peach fruits fungal communities analysis by PCR-DGGE could be applied to discriminate the farming modes. We also showed that the biological markers for each farming mode were statistically sufficient to discriminate the farming types. This global methodology is very quicker (less than 24 h) than all of other classical microbial methodologies and could be considered as a supplier for "a unique biological profile" for each farming mode. In other words, we can well note the diversity of peach fruits and any other fruits by this way.

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الملخص العربى

إستخدام البصمه الوراثيه للمجتمعات الفطرية لتحديد طريقه الزراعه باستخدام الجين التركيبى المحلل باستخدام تقنيه : 28S rDNA PCR - DGGE : تطبيـق علـى ثمـار الخـوخ

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الكشف عن نوع او طريقه الزراعة أصبح قضية مهمة لنظام التتبع للمواد الغذائيه المصدره والمستورده ، وكانت من أوائل النظريات المقدمه لتتبع مصدر المنتج الغذائى هى عن طريق تحليل عالمي للمجتمعات الميكروبية الموجوده بالطعام ومحاولة الربط الاحصائى بين الطعام وطريقه او نوع الزراعة. وقد تم الكشف عن التباين والاختلافات في تراكيب المجتمعات الفطرية لثمـار الخـوخ الاردنيـة المنشـأ من خـلال توظيف:

PCR-DGGE global technique (via 28 S rDNA analysis)

وقد قمنا بتطبيق هذه التكنولوجيا الجديدة على ثمار الخوخ الاردنية المنشأ المجمعه من نوعان مختلفان من الزراعة وهى العضوية واخرى تقليدية، كما أوضح التحليل الاحصائى للعينات عن وجود المجتمعات الفطرية منفصلة لكل نوع من انواع الزراعة. هذه التقنية هي أداة التتبع الجديدة التي تزود المواد الغذائية بأكواد بيولوجية فريدة من نوعها وتسهل لنا إمكانية تتبع الطعام بطريقة جزيئيه حديثة من طرائق الهندسة الوراثية التى تربطه بطريقه زراعته.

JOESE 5

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