Journal of Cell and Molecular Research (2011-2012) 3 (2), 43-50

Ferula assafoetida latex can cause damage to bacterial DNA

Sasan Mohsenzadeh[1*], Kolsoum Abdolahi[1] and Hassan Mohabatkar[1, 2]

[1]*Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran*
[2]*Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran*

Received 31 September 2011 Accepted 15 November 2011

Abstract

Asafoetida is the dried latex exuded from the living underground rhizome or tap root of *Ferula assafoetida*. Antibacterial characteristic of asafoetida was shown using the circular zone diameter of bacterial growth inhibition by disk-diffusion method on two gram positive and three gram negative bacteria. Then, the bacterial genomic DNA damage, induced by *F. assafoetida* latex, was demonstrated using the comparison of random amplification of polymorphic DNA profiles generated by polymerase chain reaction of control and treated bacterial genomes. The results showed that the number of primers that produced bands in each bacterium were higher in control samples compared to those treated with asafoetida. This and the absence or presence of bands between controls and treatments confirm rearrangements and DNA damage in the priming binding sites of bacterial genome.

***Keywords*:** Antibacteria, Asafoetida, DNA Polymorphism, DNA damage

Introduction

Asafoetida (Angedan in Persian), is the dried latex (gum oleoresin) exuded from the living underground rhizome or tap root of *Ferula assafoetida* (Apiaceae family), which is a perennial herb (1 to 1.5 m high). The species is native to Persia (Iran) and India. Typical asafoetida contains about 40-64% resin, 25% gum, 10-17% volatile oil, and 1.5-10% ash. The resin portion is known to contain asaresinotannol, ferulic acid, umbelliferone and four unidentified compounds (Singhal et al., 1997). Asafoetida has a broad range of uses in traditional medicine as an antimicrobial, with well documented uses for treating chronic bronchitis and whooping cough, as well as reducing flatulence (Srinivasan, 2005; Rahman et al., 2008).

The disk-diffusion method is more suitable for routine testing in a laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics. Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular zone of inhibition in which the amount of antibiotic exceeds inhibitory concentrations (Barker and Kehoe, 1995; Ottaviani et al., 2001).

∗Corresponding author E-mail: *mohsenzadeh@susc.ac.ir*

Random amplification of polymorphic DNA (RAPD) is used extensively for species classification and phylogenetic analysis (Franklin et al., 1999; Yamamoto et al., 2001; Sudupak et al., 2002; Ronimus et al., 2003; Krizova et al., 2008). A novel application of RAPD method is as biomarker assay to detect DNA damage and mutational events for example rearrangements point mutation, small insert or deletions of DNA and ploidy changes in cells of bacterium, yeast, plant, and animal (Jones and Kortenkamp, 2000; Atienzar and Jha, 2002; De Wolf et al., 2003; Atienzar et al., 2004; Castano and Becerril, 2004; El-Sherbeny et al., 2005; Shahrtash et al., 2010).

The aim of this study was to detect bacterial DNA (gram positive and gram negative) damage induced by *Ferula assafoetida* latex, using the RAPD technique. Detection of genotoxic effect involves the comparison of RAPD profiles of DNA generated by control and treated bacteria.

Materials and Methods

Microbiological culture and plant material

Five bacteria samples were taken from Persian Type Culture Collection (PTCC) of Iranian Research Organization for Science and Technology. They included three gram negative bacteria *Pseudomonas putida* (PTCC 1694), *Pseudomonas aeruginosa* (PTCC 1181) and

Xanthomonas campestris (PTCC 1473) and two gram positive bacteria *Staphylococcus aureus* (PTCC 1431) and *Bacillus subtilis* (PTCC 1156). The bacteria (100 µL of each bacterial suspension with 0.5 McFarland concentrations) were grown in petri dishes which contained a layer of agar-based Müller-Hinton growth medium. Once the growth medium in the petri dish was inoculated with the desired bacteria, the plates were incubated at the temperature of 37°C for 24 h (Atlas, 2004).

Asafoetida was obtained by abrasion of *F. assafoetida* rhizome. The plants are grown naturally in the Fars province. The asafoetida was used with 100 mg/ml concentration.

Antibacterial disk-diffusion method

The antibiotic disks (6.4 mm of diameter) that contained 10 µL of *F. assafoetida* latex (100 mg/ml concentration) or positive control antibiotics (Gentamicin against gram-negative and Penicillin against gram-positive bacteria) were placed on the agar plates streaked with bacterial suspension and inside a laminar airflow system. The antibiotic activity was measured using the circular zone diameter (mm) of bacterial growth inhibition by disk-diffusion method.

DNA extraction and RAPD experiment

Total genomic DNAs of the bacteria were extracted using GenElute Bacterial Genomic DNA kit (Sigma). The PCR amplification was carried out using twenty three 10-base pair random primers (Eurofins MWG Operon-company) with control and asafoetida treated bacterial genomic DNA as the template. PCRs were performed in a reaction mixture of 20 µl containing approximately 80 ng of the genomic DNA dissolving in sterile distilled water, 10X PCR buffer (2 µL), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 2 µl of 10 µM primer and 1 U Taq DNA polymerase. The RAPD protocol consisted of an initial denaturing step of 5 min at 94°C, followed by 35 cycles at 94°C for 1 min (denaturation), 37°C for 1 min (annealing), and 72°C for 2 min (extension) with an additional extension period of 10 min at 72°C. The PCR amplification products were separated on 1% agarose gel using Tris-Borate-EDTA (TBE) buffer and GeneRuler 100bp DNA ladder (Fermentas, Germany). All the PCR examinations were carried out by Bioer XP thermal cycler.

Results

Table 1 shows the circular zone diameter (mm) of bacterial growth inhibition by disk-diffusion method for *F. assea-fotida* latex, Gentamicin and Penicillin. The circular zone diameters of positive and negative controls were slightly higher than *F. asseafotida* latex.

Table 2 shows the nucleotide sequences of the eighty 10-mer primers which produced bands from twenty three examined primers by RAPD analysis. Table 3 is the summary of RAPD products obtained from five examined bacteria under control and asafoetida treated conditions. Total number of bands amplified from primers in each bacterium under normal and stress condition were 68 to 123. In addition, average numbers of polymorphic bands per each primer were 0.7 to 4.7 percentages. Figures 1 to 5 are RAPD profiles of genomic DNA from five examined bacteria under control and *F. assafoetida* latex treatment.

Table 1. Circular zone diameter (mm) of bacterial* growth inhibition by disk-diffusion method.

Sample	1	2	3	4	5
F. assea-fotida latex	19	15	21	18.2	17
Gentamicin#	20.3	20.1	22.1	-	-
PenicillinΦ	-	-	-	22	21

*1- *P. Putida* 2- *P. Aeruginosa* 3- *X. compestris* 4- *S. aureus* 5- *B. subtilis*
#Gentamicin as positive control against gram-negative bacteria
ΦPenicillin as positive control against gram-positive bacteria

Table 2. Nucleotide sequences of the eighty 10-mer primers* which produced bands by RAPD analysis.

Primer	Nucleotide sequence (5´-3´)
1. OPA-02	TGCCGAGCTG
2. OPA-07	GAAACCGGTG
3. OPA-08	GTGACGTAGG
4. OPA-09	GGGTAACGCC
5. OPB-07	GGTGACGCAG
6. OPB-08	GTCCACACGG
7. OPD-02	GGACCCAACC
8. OPD-03	GTCGCCGTCA
9. OPD-04	TCTGGTGAGG
10. OPD-05	TGAGCGGACA
11. OPN-01	CTCACGTTGG
12. OPN-02	ACCAGGGGCA
13. OPN-03	GGTACTCCCC
14. OPN-04	GACCGACCCA
15. OPN-06	GAGACGCACA
16. OPN-08	CCTCCAGTGT
17. OPC-14	TGCGTGCTTG
18. OPF-14	GGTGCGCACT

*All primers were provided by Eurofins MWG Operon-company (Ebersberg, Germany).

Table 3. Summary of RAPD products obtained from five examined bacteria* under control and stress.

	1		2		3		4		5	
Parameter	C	S	C	S	C	S	C	S	C	S
Total number of primers examined	23	23	23	23	23	23	23	23	23	23
Number of primers that produced bands	18	16	18	15	18	16	18	14	18	17
Total number of bands amplified from primers	86	89	123	109	120	68	117	81	106	71
Average number of bands per each primer	4.7	5.6	6.8	7.2	6.6	4.3	6.5	5.8	5.8	4.2
Total number of polymorphic bands	34.5	36	58.2	33	67.5	11	66	24	84.5	39
Average number of polymorphic bands per each primer	1.9	2.3	3.2	2.2	3.8	0.7	3.6	1.7	4.7	2.3
Polymorphic bands percentage from total bands	40.1	40.4	47.3	30.3	56.3	16.2	56.4	29.6	79.7	54.9

*1- *P. Putida* 2- *P. Aeruginosa* 3- *X. compestris* 4- *S. aureus* 5- *B. subtilis*

Discussion

In this study, the gram negative bacteria *P. putida*, *P. aeruginosa* and *X. campestris* and the gram positive bacteria *S. aureus* and *B. subtilis* were used for investigating the effect of *F. assafoetida* latex on bacterial DNA. Asafoetida has a broad range of uses in traditional antimicrobial therapy (Srinivasan, 2005) but mechanism of its function on bacteria is not clear. In this experiment, antibacterial characteristic of asafoetida was studied using the circular zone diameter (mm) of bacterial growth inhibition by disk-diffusion method and by RAPD profiles of their genomic DNA. The diameter of the inhibition zone is a function of the

amount of drug in the disk and susceptibility of the microorganism. The circular zone diameters of bacterial growth inhibition in five examined bacteria were from 15 to 21 mm which confirm high inhibition of asafoetida on bacterial growth (table 1).

Out of the 23 oligonucleotide primers tested, only 18 primers gave specific and stable results and 14 to 17 primers indicated changes in the RAPD profiles following asafoetida treatment. As table 3 shows the number of primers that produced bands in each bacterium were higher in control samples compared to the asafoetida treatment. This confirms that *Ferula assafoetida* latex may cause changes in the bacterial DNA. In addition, the comparison of RAPD profiles of genomic DNA from five bacteria between control and *F. assafoetida* latex treatment demonstrate obvious variations. Meanwhile, the primers gave a total of 970 bands ranging from 180-3000 base pairs on gel agarose electrophoresis (figure 1 to 5). Bacterial DNA damage was shown by RAPD profiles via absence or presence of bands. The total number of RAPD bands on profiles of asafoetida treatments compared to the total control bands was 276 to 143, respectively. Disappearing bands are likely to be due to changes in oligonucleotide priming sites, originated from rearrangements and DNA damage in the priming binding sites (Nelson et al., 1996; Enan, 2006; Liu et al., 2009; Shahrtash et al., 2010). Structural changes or some changes in DNA sequence due to mutation and/or large deletions (bringing two pre-existing annealing sites closer) were created in new priming sites. Our findings and some other reports support the idea that bacterial DNA polymorphisms detected by RAPD can be considered as a biomarker assay for detection of the genotoxic and DNA damage effects of natural or synthetic material with antibacterial property (Kumar et al., 2004). Previous studies had shown that changes in DNA fingerprint offered a useful biomarker assay in toxicology (Savva, 1996 and 1998).

There is no obvious relationship between the circular zone diameters of bacterial growth inhibition (table 1) and the DNA damage parameters (table 3), indicating the possible involvement of other mechanisms in the inhibition process.

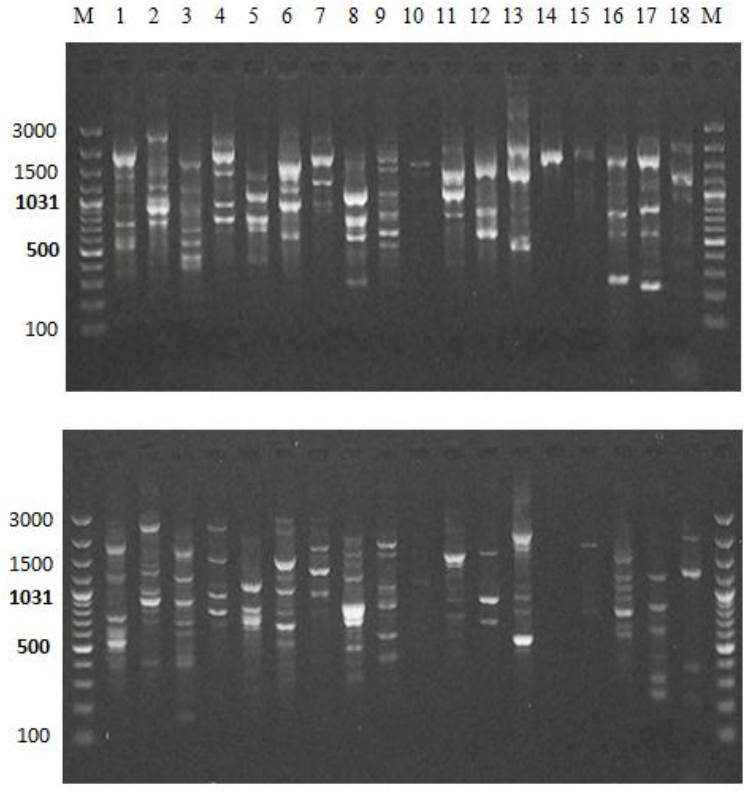

Figure 1. RAPD profiles of genomic DNA from *P. Putida*. Control (top) and *F. assafoetida* latex treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as showed in table 1.

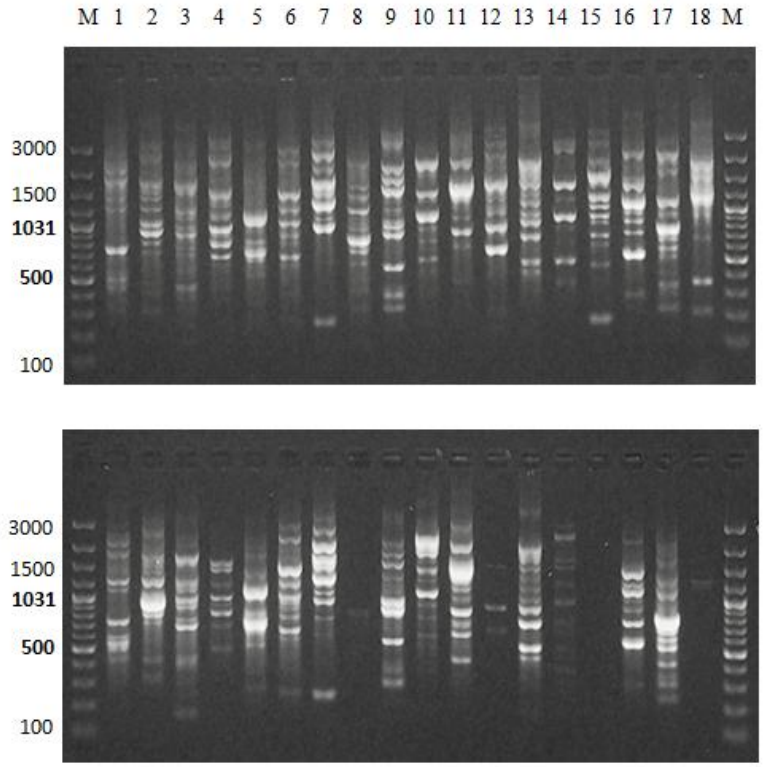

Figure 2. RAPD profiles of genomic DNA from *P. Aeruginosa*. Control (top) and *F. assafoetida* latex treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as showed in table 1.

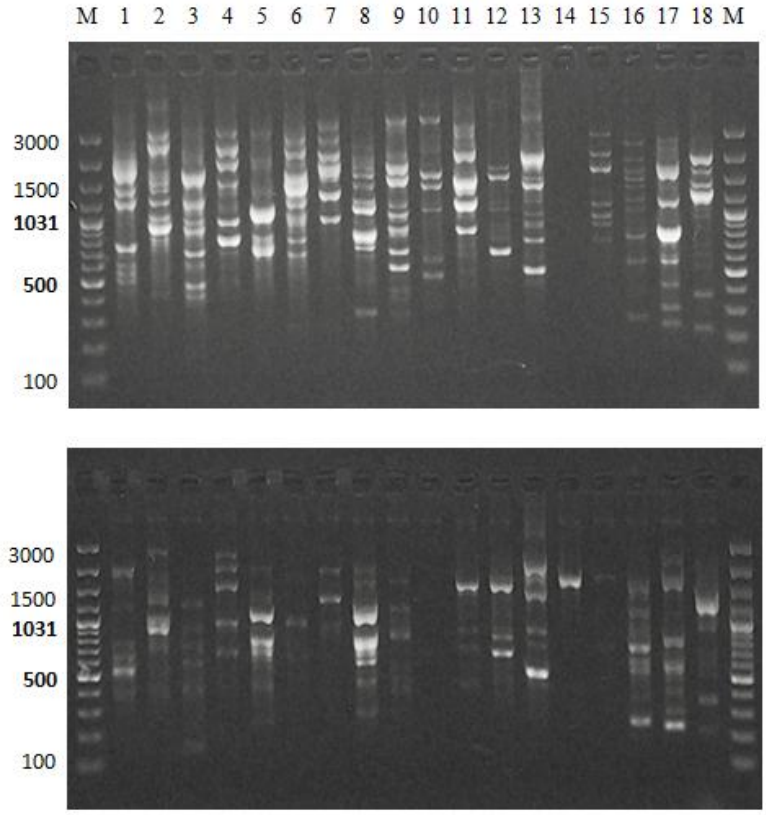

Figure 3. RAPD profiles of genomic DNA from *X. compestris*. Control (top) and *F. assafoetida* latex treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as showed in table 1.

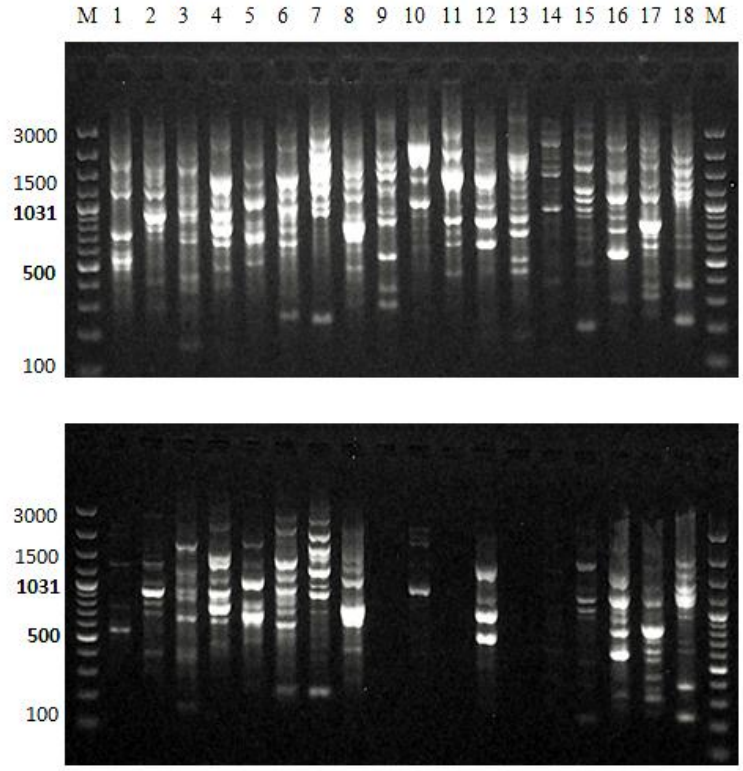

Figure 4. RAPD profiles of genomic DNA from *S. aureus*. Control (top) and *F. assafoetida* latex treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as showed in table 1.

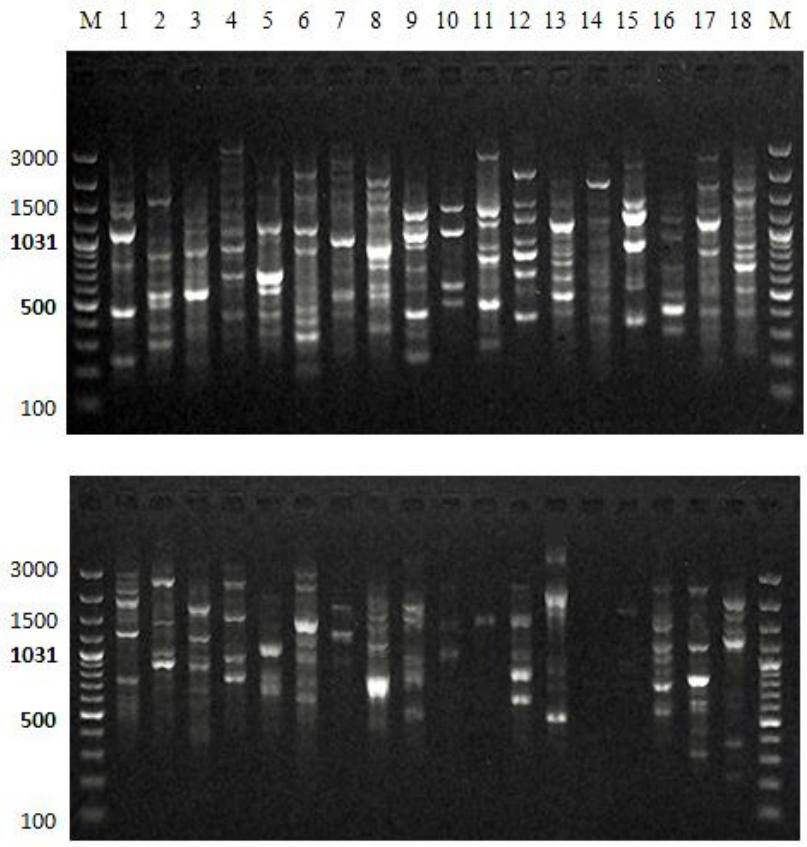

Figure 5. RAPD profiles of genomic DNA from *B. subtilis*. Control (top) and *F. assafoetida* latex treatment (down). M: 100 bp DNA ladder (100-3000) and 1 to 18 are primers as showed in table 1.

Acknowledgment

The support of this research by Shiraz University is highly acknowledged.

References

1- Atienzar F. A. and Jha A. N. (2004) The random amplified polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgenerational effects in B(*a*)P exposed *Daphnia magna*. Mutation Research 552: 125-140.

2- Atienzar F. A., Venier P., Jha A. N. and Depledge M. H. (2002) Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutation Research-Genetic Toxicology and Environmental 521: 151-163.

3- Atlas R. M. (2004) Handbook of Microbiological Media. CRC Press, London.

4- Barker G. A. and Kehoe E. (1995) Assessment of disc diffusion methods for susceptibility testing of *Aeromonas salmonicida.* Aquaculture 134: 1-8.

5- Castano A. and Becerril C. (2004) In vitro assessment of DNA damage after short and long-term exposure to benzo[*a*]pyrene using RAPD and the RTG-2 fish cell line. Mutation Research 552: 141-151.

6- Clauditz A., Resch A., Wieland K. P., Peschel A. and Götz F. (2006) Staphyloxanthin plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. Infection and Immunity 74: 4950-4953.

7- Cornelis P. (2008) Pseudomonas: Genomics and Molecular Biology. Caister Academic Press, London.

8- De Wolf H., Blust R. and Backeljau T. (2003) The use of RAPD in ecotoxicology. Mutation Research 566(3): 249-262.

9- El-Sherbeny G., Hanafy M., Hassan A. and Amin M. (2005) Rapd analysis of DNA polymorphism in the yeast *hansenula anomala* and *rhodotorula rubra* irradiated by gamma and fast neutrons. International Journal of Agriculture and Biology 6: 942-946.

10- Enan M. R. (2006) Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnology and Applied Biochemistry 43: 147-154.

11- Franklin R. B., Taylor D. R. and Mills A. L. (1999) Characterization of microbial communities using randomly amplified polymorphic DNA (RAPD). Journal of Microbiological Methods 35(3): 225-235.

12- Jones C. and Kortenkamp A. (2000) RAPD Library Fingerprinting of Bacterial and Human DNA: Applications in Mutation Detection. Teratogenesis Carcinogenesis and Mutagenesis 20: 49-63.

13- Krizova J., Spanova A. and Rittich B. (2008) RAPD and rep-PCR fingerprinting for characterization of *Bifidobacterium* species. Folia microbiologica 53: 99-104.

14- Kumar A., Tyagi M. B. and Jha P. N. (2004) Evidences showing ultra violet-B radiation-induced damage of DNA in cyanobacteria and its detection by PCR assay. Biochemical and Biophysical Research Communications 318:1025-1030.

15- Liu G. Y., Essex A., Buchanan J. T., Datta V., Hoffman H. M., Bastian J. F., Fierer J. and Nizet V. (2005) *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. The Journal of Experimental Medicine 202 (2): 209-215.

16- Liu W., Yang Y. S., Li P. J., Zhou Q. X., Xie L. J. and Han Y. P. (2009) Risk assessment of cadmium contaminated soil on plant DNA damage using RAPD and physiological indices. Journal of Hazardous Materials 161: 878-883.

17- Nakano M. M. and Zuber P. (1998) Anaerobic growth of a strict aerobe (*Bacillus subtilis*). Annual Review of Microbiology 52: 165-90.

18- Nelson J. R., Lawrence C. W. and Hinkle D. C. (1996) Thymine-thymine dimmer bypass by yeast DNA-polymerase-zeta. Science 272:1646-1649.

19- Ottaviani D., Bacchiocchi I., Masini L., Leoni F., Carraturo A., Giammarioli M. and Sbaraglia G. (2001) Antimicrobial susceptibility of potentially pathogenic halophilic vibrios isolated from seafood. International Journal of Antimicrobial Agents 18: 135-140.

20- Rahman I. R., Gul S. H. and Odhano E. A. (2008) Antimicrobial activities of *Ferula assafoetida* oil against gram positive and gram negative bacteria. American-Eurasian Journal of Agriculture 4: 203-206.

21- Ramos-González M. I., Campos M. J., Ramos J. L. and Espinosa-Urgel M. (2006) Characterization of the *Pseudomonas putida* mobile genetic element ISPpu10: an occupant of repetitive extragenic palindromic sequences. Journal of Bacteriology 188: 37-44.

22- Ronimus R. S., Parker L. E., Turner N., Poudel S., Ruckert A. and Morgan H. W. (2003) A RAPD-based comparison of thermophilic bacilli from milk powders. Int Journal of Food Microbiology 85: 46-61.

23- Savva D. (1996) DNA fingerprints as a biomarker assay in ecotoxicology. Toxicology and Ecotoxicology News 3:110-114.

24- Savva D. (1998) Use of DNA fingerprinting to detect genotoxic effects. Ecotoxicology and Environmental Safety 41: 103-106.

25- Schaad N. W., Postnikova E., Lacy G. H., Sechler A., Agarkova I., Stromberg P. E., Stromberg V. K. and Vidaver A. K. (2006) Emended classification of xanthomonad pathogens on citrus. Systematic and Applied Microbiology 29: 690-695.

26- Shahrtash M., Mohsenzadeh S. and Mohabatkar H. (2010) Cadmium-induced genotoxicity detected by the random amplification of polymorphism DNA in the maize seedling roots. Journal of Cell and Molecular Research 2: 42-48.

27- Singhal R. S., Kulkarni P. R. and Rege D. V. (1997) Handbook of indices of food quality and authenticity. Woodhead Publishing, page 395.

28- Srinivasan K. (2005) Role of spices beyond food flavoring: nutraceuticals with multiple health effects. Food Reviews International 21:167-188.

29- Sudupak M. A., Akkaya M. S. and Kence A. (2002) Analysis of genetic relationships among perennial and

annual *Cicer* species growing in Turkey using RAPD markers. Theoretical and Applied Genetics 105: 1220-1228.

30- Yamamoto K., Murakami R. and Takamura Y. (2001) Differentiation of thermophilic anaerobic gram-positive bacteria by random amplified polymorphic DNA analysis. Microbes Environments 16: 91-99.