Journal of
Applied Sciences

Differentiation of *Bacillus thuringiensis* and *Escherichia coli* by the Randomly Amplified Polymorphic DNA Analysis

[1,2]Halima Hassan Salem, [1]Tian-Hua Huang, [1,2]Bahy Ahmed Ali and [1]Qing-Dong Xie

[1]Department of Cell Biology and Medical Genetics, Shantou University, Medical College,
22 XinLing Road, Shantou, Guangdong 515031, People's Republic of China
[2]Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research
Institute, Mubarak City for Scientific Research and Technology Applications,
New Borg El-Arab City 21934, Alexandria, Egypt

Abstract: Random Amplified Polymorphic DNA (RAPD) is a method of producing a biochemical fingerprint of a particular species. This study involved the isolation of DNA from three *Bacillus thuringiensis* (*B. thuringiensis*) [*B. thuringiensis tenebrionis* (tt4) and *B. thuringiensis israelensis* (i977)] as reference strains and new isolate *B. thuringiensis* strain (66) using the DNeasy Mini Kit (Qiagen, Inc. protocol). Two *Escherichia coli* (*E. coli* BL21 and *E. coli* C1a) DNAs are provided as components of RAPD Analysis Kit. RAPD analysis was performed using Ready-To-Go™ Analysis Beads and nine primers. Bands were then analyzed using a binary system and a phylogenetic tree created. The five strains segregated into two major clusters. The maximum linkage distance between the two major clusters was 103 units. First cluster consisted of two *E. coli* strains and the second cluster consisted of the three *B. thuringiensis* strains based on genetic similarity in their RAPD profiles. The *B. thuringiensis* reference strains were located in subcluster showing high similarity. Genetic similarities estimated as band sharing were calculated and all comparisons among bacterial strains have been done. The results showed that RAPD-PCR is powerful method for differentiation between *B. thuringiensis* and *E. coli*.

Key words: *Bacillus thuringiensis*, *Escherichia coli*, RAPD-PCR

INTRODUCTION

Bacillus is a large genus of bacteria which are able to form spores in the presence of oxygen. *Bacillus* is common in soil, manure and plant material. *Escherichia* is most common of the inhabitants of intestinal tract is a facultative anaerobe. *E. coli*, used as an important tool for basic research, is one of the most widely studied enteric bacteria. Its presence in food or water is used as an indicator of fecal contamination.

RAPD-PCR is means of creating a biochemical fingerprint of an organism. This technology is proving to be quite useful in typing strains of bacteria involved in nosocomial (hospital acquired) outbreaks of infectious disease. Genetic typing of bacteria by PCR amplification of variable DNA has been described for a large series of species (Naffa *et al.*, 2006; Seppola *et al.*, 2006; Abdul Manaf *et al.*, 2006; Tankson *et al.*, 2006; Aymerich *et al.*, 2006; Catzeddu *et al.*, 2006; Gomes *et al.*, 2005; Naureen *et al.*, 2005; Fujii *et al.*, 2005; Duarte *et al.*, 2005; Yin *et al.*, 2005; De la Puente-Redondo *et al.*, 2000; Gaviria Rivera and Priest, 2003; Van Belkum, 1994; Bingen *et al.*, 1993; Brousseau *et al.*, 1993; Czajka *et al.*, 1993; De Bruyn, 1992; Fekete *et al.*, 1992; Hennessy *et al.*, 1993; Jayarao *et al.*, 1992; Mazurier *et al.*, 1992; Mazurier and Wernars, 1992; Menard *et al.*, 1992; Meyers *et al.*, 1993; Ralph *et al.*, 1993; Ross and Dwyer, 1993; Struelens *et al.*, 1993; Welsh *et al.*, 1992; Wilson *et al.*, 1992; Woods *et al.*, 1992).

RAPD technology has been particularly used for genetic and molecular studies as it is a simple, powerful and rapid method. During the last few years, we used RAPD for a wide range of applications in different organisms (Ali *et al.*, 2002, 2003; 2003a, b; 2004a, b; Soliman *et al.*, 2003; Ali and Ahmed, 2001; Ahmed *et al.*, 2004, El-Manhaly *et al.*, 2004; El-Zaeem *et al.*, 2006; Salem *et al.*, 2005).

Corresponding Author: Dr. Bahy Ahmed Ali, Associate Professor, Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Technology Applications, New Borg El-Arab City 21934, Alexandria, Egypt

In this study, RAPD-PCR technique was employed to differentiate between two different genera (*Bacillus* and *Escherichia*) including three *B. thuringiensis* strains (two reference strains and new isolate) and two *E. coli* strains.

MATERIALS AND METHODS

Strains: Two *B. thuringiensis* strains [*B. thuringiensis tenebrionis* (tt4) and *B. thuringiensis israelensis* (i977)] were kindly supplied by Prof. Priest, FG, Heriot University, England) and used as reference strains. The new *B. thuringiensis* isolate (designated 66) was recovered from dead *Biomphalaria alexandrina* snails in Egypt and selected from several isolates on the basis of bioassay of its toxicity against *Biomphalaria alexandrina* snails, in addition to sequence of morphological, biochemical and molecular tests (Salem, 2004).

DNA extraction: Genomic DNA was extracted from the three *B. thuringiensis* strains using DNeasy Mini Kit (Gene Company, Limited, Guangzhou, China). Two *E. coli* (BL21 and C1a) DNAs are provided as controls to assay the ability of Ready-To-Go™ RAPD Analysis Kit (Guangzhou Amsure Trade Ltd., China).

RAPD-PCR primers: A total of nine random sequence primers that were 9-10 bases long were used in this study (Table 1). Primer 2 (P2) is a package as a component of Ready-To-Go™ RAPD Analysis Kit (Guangzhou Amsure Trade Ltd., China). Other primers were synthesized by Shanghai DNA Biotechnologies Co., Ltd, Shanghai, China.

PCR conditions: DNA amplification was performed using Ready-To-Go™ RAPD Analysis Beads (27-9500-01, Guangzhou Amsure Trade Ltd., China) according to the manufacturer's description. The amplification reactions were performed in 25 μL volumes. From 10-15 ng template DNA and 25 pmol of a single RAPD primer were added to the tube of RAPD analysis Beads. The mixture was heated to 95°C for 5 min followed by 45 cycles consisting of 95°C for 1 min, 36°C for 1 min and 72°C for 2 min and finally 72°C for 3 min in a Peltier Thermal Cycler (PTC-200, MJ Research Inc., Watertown, USA). After amplification, the banding pattern of the randomly amplified DNA was fractionated by electrophoresis on 1.1% agarose gel and run at 60 V in 1X TBE buffer containing 0.5 μg ethidium bromide per mL. Gels were imaged with the gel documentation system (UVP, USA).

Table 1: Nucleotide sequence of RAPD primers

Primers	Nucleotides sequences (5'-3')	GC(%)	Reference
P1	GGT GCG GGA A	70.00	Nilsson *et al.* (1998)
P2	GTT TCG CTC C	60.00	
P3	GTA GAC CCG T	60.00	
P4	AAG AGC CCG T	60.00	Ready-To-Go
P5	AAC GCG CAA C	60.00	RAPD Analysis
P6	CCC GTC AGC A	70.00	Kit (27-9502-01),
			Amersham Biosciences
P7	CCG AGT CCA	66.70	
P8	ACG CGC CCT	77.80	
P9	CCG GCG GCG	100.00	

RAPD data analysis: The RAPD bands were scored for their presence (1) or absence (0). The index of similarity among strains was calculated using the formula: $Bab = 2Nab/(Na+Nb)$, where Nab is the number of common fragments observed in individuals a and b and Na and Nb are the total number of fragments scored in a and b, respectively (Lynch, 1990). The similarity index was calculated for each primer separately and the average for all primers was carried out with each comparison. Moreover, the scored band data (Presence or absence) was subjected to cluster analysis using Statistica/W5.0 Software package (StatSoft Inc., Tulsa, OK, USA). The Dendrogram was constructed by Ward's method of clustering using minimum variance algorithm. The dissimilarity matrix was developed using Squared Euclidean Distance (SED).

RESULTS

All nine RAPD primers yielded reproducible DNA profiles. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions. Most of the primers were successfully amplified polymorphic bands among the five strains of the two genera (Fig. 1-3).

A phylogenetic tree was generated from RAPD patterns of the five strains (Fig. 4). The five strains are grouped into two major clusters A and B. Cluster A consisted of two *E. coli* strains and cluster B consisted of the three *B. thuringiensis* strains based on genetic similarity in their RAPD profiles. The maximum linkage distance between the two major clusters was 103 units. Cluster A was grouped into two subclusters A1 and A2 one for each *E. coli* strain (BL21, C1a). The second cluster (B) was grouped into two subclusters (B1 and B2) and consisted of three *B. thuringiensis* strains, which showed less variation than the first cluster. The maximum linkage distance between the two subclusters (B1 and B2) was 55 units. Subcluster B1 consisted of only the new isolate (66). While, subcluster B2 was grouped into two subclusters (B2.1 and B2.2) and consisted of two reference strains (tt4 and i977).

Table 2: Genetic similarity estimated for each primer among the five bacterial strains

| Comparisons	Primers									
	P1	P2	P3	P4	P5	P6	P7	P8	P9	Average
BL21 × C1a	66.67	33.33	75.0	100.0	100.0	100.0	00.00	83.33	85.71	71.56
BL21 × 66	40.00	25.00	28.57	46.15	66.67	33.33	25.00	16.66	28.57	31.26
BL21 × tt4	20.00	25.00	28.57	18.81	40.00	16.67	20.00	42.86	22.22	26.01
BL21 × i977	20.00	33.33	44.44	30.77	33.33	00.00	00.00	16.67	00.00	19.84
C1a × 66	33.33	33.33	57.14	46.15	66.67	33.33	00.00	16.66	36.36	35.88
C1a × tt4	25.00	60.00	28.57	28.57	40.00	16.67	50.00	28.57	00.00	30.82
C1a × i977	25.00	85.71	22.22	28.57	33.33	00.00	33.33	16.67	00.00	27.20
66 × tt4	00.00	60.0	33.33	54.54	57.14	83.33	33.33	71.42	66.67	51.08
66 × i977	00.00	28.57	50.00	54.54	50.00	54.54	00.00	50.00	66.67	39.37
tt4 × i977	100.0	66.67	75.00	100.0	85.71	72.73	40.00	71.73	80.00	76.87
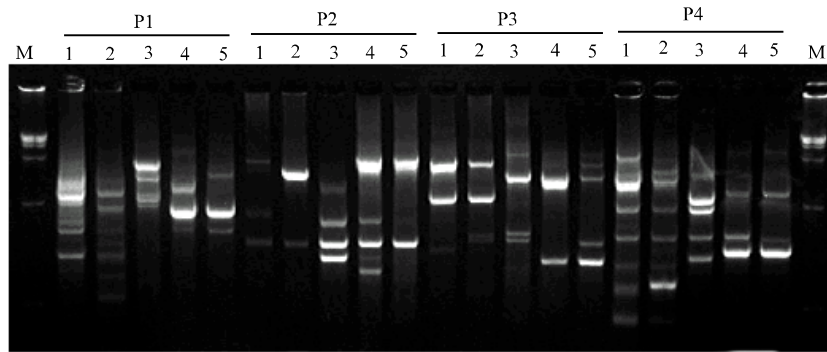
Fig. 1: RAPD fingerprints profiles of five strains obtained with four primers (P1-P4), Lanes M: DNA marker (DL 15 Kb); Lanes 1-5: Strains (BL21, Cla, 66, tt4 and i977, respectively)
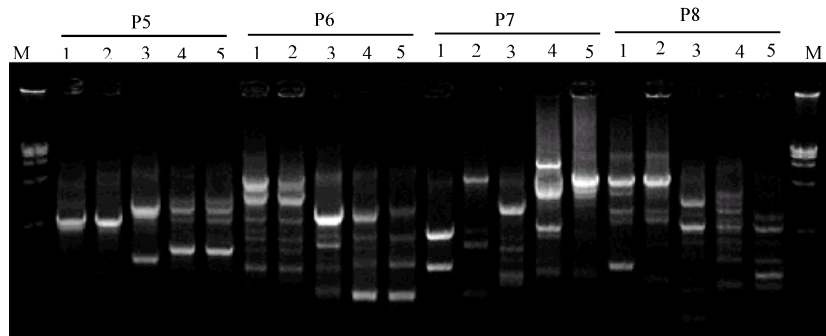

Fig. 2: RAPD fingerprints profiles of five strains obtained with four primers (P5-P8), Lanes M: DNA marker (DL 15 Kb); Lanes 1-5: Strains (BL21, Cla, 66, tt4 and i977, respectively)

Data presented in Table 2 showed that the genetic similarity estimated as band sharing (BS) among different bacterial strains. Among primers used in this study, primers (P4, P5 and P6) showed the highest similarity (100%) between *E. coli* strains (BL21, Cla). While, the highest similarity (100%) between the *B. thuringiensis* strains (tt4 and i977) was obtained with primers (P1 and P4). On the other hand and based on RAPD data of the nine primers, the average of genetic similarities between the new isolate (66) and BL21; Cla; i977 and tt4 were 31.26; 35.88; 39.37 and 51.08%, respectively.

DISCUSSION

RAPD-PCR has proved to be an informative method suitable for the study of a large number of strains in a short time. RAPD is a well-established easy method used to classify closely related strains (Williams *et al.*, 1990; Ghelardi *et al.*, 2002; Pinchuk *et al.*, 2002). We tested the ability of this method to differentiate between five bacterial strains from tow different genera. To ensure that the amplified DNA bands originated from genomic DNA and not primer artifacts, negative control was carried out
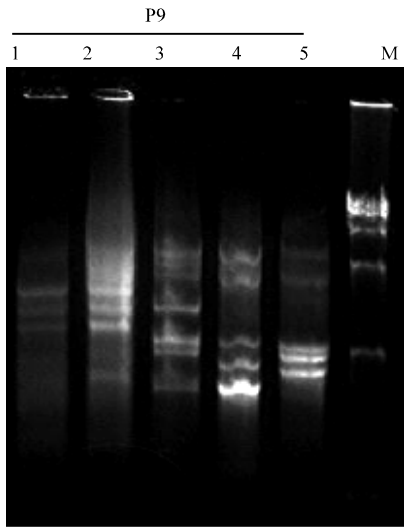
P9

Fig. 3: RAPD fingerprints profiles of five strains obtained with primer 9 (P9), Lanes M: DNA marker (DL 15 Kb); Lanes 1-5: Strains (BL21, Cla, 66, tt4 and i977, respectively)
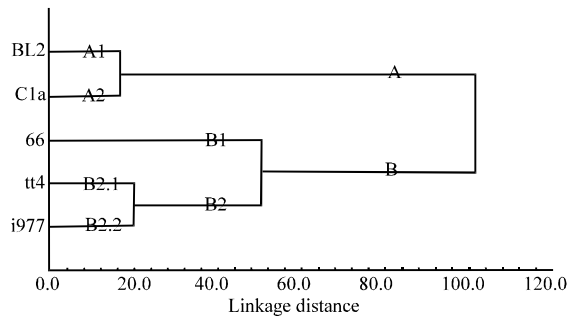

Fig. 4: Phylogenetic tree built on the bases on RAPD profiles of five strains using all primers

for each primer/strain combination. No amplification was detected in control reactions. On other hand, the GC% of primers used in this study ranged from 60 to 100% and the results showed that GC content is not essential requirements for generation of polymorphic bands. This result is in agreement with the findings of other researchers (Gomes *et al.*, 2005; Alos *et al.*, 1993; Brikun *et al.*, 1994; Cave *et al.*, 1994). On the contrary, it has been observed that random primers with high GC content (>60%) resulted in a greater and better reproducible number of strain specific bands in enterotoxigenic *E. coli* (Akopyanz *et al.*, 1992; Makino *et al.*, 1994; Pacheco *et al.*, 1996). In general, RAPD patterns provide a convenient and simple method for bacterial typing with reasonably reproducible results so long as care is taken with the concentrations of template DNA (Davin-Regli *et al.*, 1995) and magnesium

in the reaction (Park and Kohel, 1994). The arbitrary primers recognize differences in the prevalence and positions of annealing sites in genome producing sets of fragments that are considered to reflect the genomic composition of the strain (Gaviria-Rivera and Priest, 2003).

A phylogenetic tree (Fig. 4) was generated from the diverse five strains RAPD patterns and different primers were highly suggestive of a genetically diverse population. The placement of the two *E. coli* (BL21 and C1a) and three *B. thuringiensis* strains each in one major cluster indicated the effectiveness of RAPD-PCR technique as powerful method to differentiate between bacterial strains from different genera. Moreover, the placement of BL21 and C1a in on subcluster demonstrated the highest genetic similarity between them. Also, results showed that the closer proximity of the new isolate (66) to tt4 and i977 (51.08 and 39.37%, respectively) and the farthest from BL21 and C1a (31.26 and 30.82%, respectively). The RAPD study presented here indicated that it could provide an alternative to Serotyping for *B. thuringiensis*. Serotyping has provided a valuable subspecific classification of *B. thuringiensis* for over four decades but suffers limitations (Gaviria-Rivera and Priest, 2003). Some strains cannot be typed because they lack flagella or autoagglutinate and specialist antisera are needed. Moreover, typing based on whole genome patterns of one kind or another has become the norm for medically important bacteria (Gurtler and Mayall, 2001).

ACKNOWLEDGMENTS

REFERENCES

Abdul Manaf, S.R., S. Mustafa, N.M. Amin and A.M. Ali, 2006. Genetic relatedness among isolates of *Acanthamoeba* based on RAPD analysis. J. Applied Sci., 6: 15-19.

Ahmed, M.M.M., B.A. Ali and S.Y. El-Zaeem, 2004. Application of RAPD markers in fish: Part I-some genera (*Tilapia, Sarotherodon* and *Oreochromis*) and species (*Oreochromis aureus* and *Oreochromis niloticus*) of Tilapia. Intl. J. Biotechnol., 6: 86-93.

Akopyanz, N., N.O. Bukanov., T.V. Westblom, S. Kresovich and D.E. Berg, 1992. DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based fingerprinting. Nucleic Acids Res., 20: 5137-5142.

Ali, B.A. and M.M.M. Ahmed, 2001. Random amplified polymorphic DNA in some chicken strains. Proceeding of the Congress of Role of Biochemistry in Environment and Agriculture. Cairo University, Cairo, (Egypt), Part I, pp: 23-31.

Ali, B.A., M.M.M. Ahmed, M. Bahie El-Deen and H.M. Shalan, 2002. Genetic variability in the 17th generation of Japanese quail selected for high eggs and meat production. Egypt. Poult. Sci. J., 22: 59-71.

Ali, B.A., 2003a. Detection of DNA alteration in abnormal phenotype of broiler chicken male by random amplified polymorphic DNA (RAPD). Afr. J. Biotechnol., 2: 153-156.

Ali, B.A., 2003b. Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers. Afr. J. Biotechnol., 2: 194-197.

Ali, B.A., M.M.M. Ahmed and O.M. Aly, 2003. Relationship between geneticsimilarity and some productive traits in local chicken strains. Afr. J. Biotechnol., 2: 46-47.

Ali, B.A., M.M.M. Ahmed and S.Y. El-Zaeem, 2004a. Application of RAPD markers in fish: Part II-Among and within families; *Cichlidae* (freshwater), *Mugilidae* (Catadromus), *Sparidae* and *Serranidae* (marine). Intl. J. Biotechnol., 6: 393-401.

Ali, B.A., T.H. Huang, D.N. Qin and X.M. Wang, 2004b. A review of random amplified polymorphic DNA (RAPD) markers in fish. Rev. Fish Biol. Fish, 14: 4443-4453.

Alos, J.I., T. Lambert and P. Courvalin, 1993. Comparison of two molecular methods for tracing Nosocomial transmission of *Escherichia coli* K in 1 a neonatal unit. J. Clin. Microbiol., 31: 1704-1709.

Aymerich, T., B. Martin, M. Garriga, M.C. Vidal-Carou, S. Bover-Cid and M. Hugas, 2006. Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. J. Applied Microbiol., 100: 40-49.

Bingen, E., C. Boissinot, P. Desjardins, H. Cave, N. Brahimi, N. Lambert, E. Denamur, P. Blot and J. Elion, 1993. Arbitrarily primed PCR provides rapid differentiation of *Proteus mirabilis* isolates from a pediatric hospital. J. Clin. Microbiol., 31: 1055-1059.

Brikun, I., K. Suziedelis and D.E. Berg, 1994. DNA sequence divergence among *Escherichia coli* K-12 detected by arbitrary primer PCR fingerprinting. J. Bacteriol., 176: 1673-1682.

Brousseau, R., A. Saint-Onge, G. Prefontaine, L. Masson and J. Cabana, 1993. Arbitrary primer polymerase chain reaction, a powerful method to identify *Bacillus thuringiensis* serovars and strains. Applied Environ. Microbiol., 59: 114-119.

Catzeddu, P., E. Mura, E. Parente, M. Sanna and G.A. Farris, 2006. Molecular characterization of lactic acid bacteria from sourdough breads produced in Sardinia (Italy) and multivariate statistical analyses of results. Syst. Applied Microbiol., 29: 138-144.

Cave, H., E. Bingen., J. Elion and E. Denamur, 1994. Differentiation of *Escherichia coli* strains using randomly amplified polymorphic DNA analysis. Res. Microbiol., 145: 141-150.

Czajka, J., N. Bsat, M. Piani, W. Russ, K. Sultana, M. Wiedmann, R. Whitaker and C.A. Batt, 1993. Differentiation of *Listeria monocytogenes* and *Listeria innocua* by 16S rRNA genes and intraspecies discrimination of *Listeria monocytogenes* strains by random amplified polymorphic DNA polymorphisms. Applied Environ. Microbiol., 59: 304-308.

Davin-Regli, A., A.R.N. Charrel, C. Bollet and P. de Micco, 1995. Variations in DNA concentration significantly affect the reproducibility of RAPD fingerprint patterns. Res. Microbiol., 146: 561-568.

De Bruyn, F.J., 1992. Use of repetitive (REP and ERIC) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* and other soil bacteria. Applied Environ. Microbiol., 58: 2180-2187.

De la Puente-Redondo, V.A., N. García del Blanco, C.B. Gutiérrez-Martín, F.J. García-Peña and E.F. Rodríguez-Ferri, 2000. Comparison of different PCR approaches for typing of *Francisella tularensis* strains. J. Clin. Microbiol., 38: 1016-1022.

Duarte, R.S., R.R. Barros, R.R. Facklam and L.M. Teixeira, 2005. Phenotypic and genotypic characteristics of *Streptococcus porcinus* isolated from human sources. J. Clin. Microbiol., 43: 4592-4601.

El-Manhaly, M.A., M.S. Saleh, S.A., N.S.A. Ghura, M.M.M. Ahmed and B.A. Ali, 2004. Identification of three Egyptian sugar beet genotypes and detection of DNA similarity. 67th IIRB Congress-Feb. 11-12, Brussels, (Belgium).

El-Zaeem, S.Y., B.A. Ali and M.M.M. Ahmed, 2006. Random amplified polymorphic DNA finger print and genetic similarity among four genera and between two phenotypes of cultured carps in Egypt. Intl. J. Agric. Biol., 8: 111-115.

Fekete, A., J.A. Bantle, S.M. Hailing and R.W. Stich, 1992. Amplification fragment length polymorphism in *Brucella* strains by use of polymerase chain reaction with arbitrary primers. J. Bacteriol., 174: 7778-7783.

Fujii, T., K. Nakashima and N. Hayashi, 2005. Random amplified polymorphic DNA-PCR based cloning of markers to identify the beer-spoilage strains of *Lactobacillus brevis*, *Pediococcus damnosus*, *Lactobacillus collinoides* and *Lactobacillus coryniformis*. J. Applied Micobiol., 98: 1209-1220.

Gaviria-Rivera, A.M. and F.G. Priest, 2003. Molecular typing of *Bacillus thuringiensis* serovars by RAPD-PCR system. Applied Microbiol., 26: 254-261.

Ghelardi, E., F. Celandroni, S. Salvetti, C. Barsotti, A. Baggiani and S. Senesi, 2002. Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two foodpoisoning outbreaks. FEMS Microbiol. Lett., 208: 129-134.

Gomes, A.R., L. Muniyappa, G. Krishnappa, V.V.S. Suryanarayana, S. Isloor, B. Prakash and P.G. Hugar, 2005. Genotypic characterization of avian *Escherichia coli* by random amplification of polymorphic DNA. Intl. J. Poult. Sci., 4: 378-381.

Gurtler, V. and B.C. Mayall, 2001. Genomic approaches to typing, taxonomy and evolution of bacterial isolates. Intl. J. Syst. Evol. Microbiol., 51: 3-16.

Hennessy, K., J.J. landolo and B.W. Fenwick, 1993. Serotype identification of *Actinobacillus pleuropneumoniae* by arbitrarily primed PCR. J. Clin. Microbiol., 31: 1155-1159.

Jayarao, B.M., B.J. Bassam, G. Caetano-Anolles, P.M. Gresshoff and S.P. Oliver, 1992. Subtyping of *Streptococcus uberis* by DNA amplification fingerprinting. J. Clin. Microbiol., 20: 1347-1350.

Lynch, M., 1990. The similarity index and DNA fingerprinting. Mol. Biol. Evol., 7: 478-484.

Makino, S., Y. Okada, T. Maruyama, S. Kaneko and C. Sasakawa, 1994. PCR-based random amplified polymorphic DNA fingerprinting of *Yersinia pseudotuberculosis* and its practical applications. J. Clin. Mirobiol., 32: 65-69.

Mazurier, S., A. Audurier, N. Marquet-Van der Meer, S. Notermans and K. Wernars, 1992. A comparative study of randomly amplified polymorphic DNA analysis and conventional phage typing for epidemiological studies of *Listeria monocytogenes* isolates. Res. Microbiol., 143: 507-512.

Mazurier, S. and K. Wernars, 1992. Typing of *Listeria strains* by random amplification of polymorphic DNA. Res. Microbiol., 143: 499-505.

Menard, C., R. Brousseau and C. Muton 1992. Application of polymerase chain reaction with arbitrary primer (AP-PCR) to strain identification of *Porphyromonas* (Bacteroides) *gingivalis*. FEMS Microbiol. Lett., 74: 163-168.

Meyers, L.E., S.V.P.S. Silva, J.D. Procunier and P.B. Little, 1993. Genomic fingerprinting of *Haemophilus somnus* isolates by using a random-amplified polymorphic DNA assay. J. Clin. Microbiol., 31: 512-517.

Naffa, R.G., S.M. Bdour, H.M. Migdadi and A.A. Shehabi, 2006. Enterotoxicity and genetic variation among clinical *Staphylococcus aureus* isolates in Jordan. J. Med. Microbiol., 55: 183-187.

Naureen, Z., S. Yasmin, S. Hameed, K.A. Malik and F.Y. Haeez, 2005 . Characterization and screening of bacteria from rhizosphere of maize grown in Indonesian and Pakistani soils. J. Basic. Microbiol., 45: 447-459.

Nilsson, J., B. Svensson, K. Ekelund and A. Christiansson, 1998. A RAPD-PCR method for large-scale typing of *Bacillus cereus*. Lett. Applied Microbiol., 27: 168-172.

Pacheco, A.B.F., B.E.C. Guth, D.F. DeAlmeida and L.C.S Ferreira, 1996. Characterization of enterotoxigenic *Escherichia coli* by random amplification of polymorphic DNA. Res. Microbiol., 147: 175-182.

Park, Y.H. and R.J.b. Kohel, 1994. Effect of concentration of $MgCl_2$ on random-amplified DNA polymorphism. BioTechniques, 16: 652-655.

Pinchuk, I.V., P. Bressollier, I.B. Sorokulova, B. Verneuil and M.C. Urdaci, 2002. Amicoumacin antibiotic production and genetic diversity of *Bacillus subtilis* strains isolated from different habitats. Res. Microbiol., 153: 269-276.

Ralph, D., M. McClelland, J. Welsh, G. Baranton and P. Perolat, 1993. *Leptospira* species categorized by arbitrarily primed polymerase chain reaction (PCR) and by mapped restriction polymorphisms in PCR-amplified rRNA genes. J. Bacteriol., 175: 973-981.

Ross, B.C. and B. Dwyer, 1993. Rapid, simple method for typing plates of *Mycobacterium tuberculosis* by using the PCR. J. Clin. Microbiol., 31: 329-334.

Salem, H.H., 2004. Attempts for genetic improvement of *Bacillus thuringiensis* strain as biocontrol agent for bilharzian snails. Ph.D Thesis, Benha, Zagazig University, Zagazig, (Egypt).

Salem, H.H., B.A. Ali, T.H. Huang and D.N. Qin, 2005. Use of randomly amplified polymorphic DNA (RAPD) markers in poultry research. Intl. J. Poult. Sci., 4: 804-811.

Seppola, M., R.E. Olsenb, E. Sandakera, P. Kanapathippillaic, W. Holzapfeld and E. Ringø, 2006. Random amplification of polymorphic DNA (RAPD) typing of carnobacteria isolated from hindgut chamber and large intestine of Atlantic cod (*Gadus morhua* l.). Syst. Applied Microbiol., 29: 131-137.

Soliman, S.S., B.A. Ali and M.M.M. Ahmed, 2003. Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. Afr. J. Biotechnol., 2: 86-87.

Struelens, M., E. Carlier, N. Maes, E. Serruys, W. Quint and A. van Belkum, 1993. Nosocomial colonisation and infection with multiresistant *Acinetobacter baumannii*: Outbreak delineation using DNA macrorestriction analysis and PCR fingerprinting. J. Hosp. Infect., 25:15-32.

Tankson, J.D., P.J. Fedorka-Cray, C.R. Jackson and M. Headrick, 2006. Genetic relatedness of a rarely isolated Salmonella: *Salmonella enterica* serotype Niakhar from NARMS animal isolates. J. Antimicrob. Chemother., 57:190-198.

Van Belkum, A., 1994. DNA fingerprinting of medically important microorganisms by use of PCR. Clin. Microbiol. Rev., 7: 174-184.

Welsh, J., C. Pretzman, D. Postic, I. Saint-Girons, G. Baranton and M. McClelland, 1992. Genomic fingerprinting by arbitrarily primed polymerase chain reaction resolves *Borrelia burgdorferi* to three distinct phyletic groups. Intl. J. Syst. Bacteriol., 42: 370-377.

Williams, G.K.J., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Wilson, M.A., R.B. Rinker and L.J. Hoffman, 1992. Comparison of DNA fingerprints and somatic serotypes of serogroup B and E *Pasteurella multocida* isolates. J. Clin. Microbiol., 30:1518-1524.

Woods, C.R., J. Versalovic, T. Koeuth and J.R. Lupski, 1992. Analysis of relationships among isolates of *Citrobacter diversus* by using DNA fingerprints generated by repetitive sequence-based primers in the polymerase chain reaction. J. Clin. Microbiol., 30: 2921-2929.

Yin, Y.N., Y.F. Chen, S.M. Li and J.H. Guo, 2005. RAPD analysis of plant pathogenic coryneform bacteria. Wei Sheng Wu Xue Bao, 45: 837-841.