

ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)



Bio Technology



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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Influence of Thermal Cyclers on Day-to-Day Reproducibility of Random Amplified Polymorphic DNA Fingerprints

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Abstract: The day-to-day reproducibility of random amplified polymorphic DNA (RAPD) fingerprints was tested using two different thermal cyclers. Ten different oligonucleotide primers were used to obtain patterns of amplified fragments from one DNA sample (*Bacillus thuringiensis*) isolate. Experiments were repeated under same conditions five times. Results showed that the RAPD fingerprints of each primer in the same time by using different thermal cyclers gave evidence for excellent reproducibility. Among primers used in this study, 3 primers (p2, p3 and p5) gave evidence for good day-to-day reproducibility. However, other primers (p1, p4 and p6-10) revealed that the day-to-day reproducibility was poor and RAPD fingerprint variations (appearance or disappearance of bands) occurred.

Key words: RAPD reproducibility, thermal cycler, DNA fingerprints

INTRODUCTION

Randomly amplified polymorphic DNA (RAPD) is a technique based on the PCR using primers of arbitrary sequence. RAPD uses small quantities of DNA; no sequence information is needed (Morgan *et al.*, 1995). RAPD has been used increasingly for taxonomic identification, isolate differentiation and phylogenetic relationship in different organisms (Hadrys *et al.*, 1992; Welsh *et al.*, 1992; Williams *et al.*, 1993; Deng and Cliver, 1998; Salem *et al.*, 2006; Ali *et al.*, 2004a; Ahmed *et al.*, 2004; El-Manhaly *et al.*, 2004; El-Zaeem *et al.*, 2006). Recently, genetic typing of bacteria has been described for a large series of species (Naffa *et al.*, 2006; Seppolaa *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).

The RAPD method is increasingly used due to its ease of operation, although constant references are made to the fact that reproducibility between laboratories becomes almost impossible due to the method's dependence on the thermal cycler and type of *Taq* DNA polymerase used (Meunier and Grimont, 1993; Tyler *et al.*, 1997; Hayford *et al.*, 1999). Earlier reproducibility studies done on these methods (Welsh and McClelland, 1990; Bassam *et al.*, 1992) considered the following parameters:

template DNA and primer concentration, *Taq* DNA polymerase concentration, temperature of annealing, number of thermal cycles and MgCl₂ concentration. The influence of other parameters, including the *Taq* DNA polymerase manufacturer and the type of PCR apparatus used, upon the reproducibility of RAPD fingerprints was reported by Meunier and Grimont (1993). On the other hand, the method appears to have some merit when it comes to generating genetic markers for linkage mapping and creating species-specific probes (Fani *et al.*, 1993; Hadrys *et al.*, 1992; Quere *et al.*, 1997) when no sequence data are available for the genome in question. The reproducibility, other limitations and advantages of RAPD fingerprints have been discussed in our previous review articles (Ali *et al.*, 2004b; Salem *et al.*, 2005).

The objective of the present study was to test the effect of two different thermal cyclers including three blocks on day-to-day reproducibility of RAPD fingerprints.

MATERIALS AND METHODS

Experiments of this study were carried out in laboratory of Cell Biology and Medical Genetics Department, Shantou University, Medical College, People's Republic of China during April 2006.

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Table 1: Nucleotide sequence of RAPD primers used in this study

Primers	Nucleotides Sequences (5'-3')	GC%	Reference
P1	CCG AGT CCA	66.7	Nilsson <i>et al.</i> (1998)
P2	ACG CGC CCT	77.8	
P3	CCG GCG GCG	100	
P4	CGG CCA CTG T	70	
P5	ACG TAT CTGC	50	Meunier and Grimont (1993)
P6	CGG CCC CTG T	80	
P7	GTT TCC GCC C	70	Vettori <i>et al.</i> (1996)
P8	CGG CCT CTG C	80	Stephan (1996)
P9	ACA ACT GCT C	50	
P10	ACG TAT CTG C	50	

Genomic DNA: Genomic DNA was extracted from new *B. thuringiensis* isolate (designated 66) which recovered from dead *Biomphalaria alexandrina* snails in Egypt (Salem, 2004), using DNeasy Mini Kit (Gene Company, Limited, Guangzhou, China). The concentration of DNA and its relative purity was determined using UV spectrophotometer based on absorbance at 260 and 280 nm, respectively.

RAPD-PCR primers: A total of ten random sequence primers that were 9-10 bases long were used in this study (Table 1). All primers were synthesized by Shanghai DNA BioTechnologies Co., Ltd, Shanghai, China.

RAPD reaction: A 25 µL PCR contained 12 ng as template DNA and 25 pmol of a single RAPD primer was performed. Each sample of the 10 primers was performed in triplicate as following: reaction mix (3 volumes) was prepared into

0.2 mL tube and three volumes of sample DNA (36 ng) were added and mixed to give a final volume of 75 µL. This volume was divided into 3 tubes 25 µL each (lanes 1A, 1B and 2). The amplification of the three samples from each primer (p1-p10) was performed using different wells of same thermal cycler' blocks from day to day (Fig. 1).

Thermal cyclers: Two different PCR thermal cyclers (MJ Research Inc., Watertown, USA) were used in the present study: 1) MJR PTC-200 with a dual 48-well block (1A and 1B) and 2) MJR PTC-100 is a 96-well block (Fig. 1). The PCR cycling parameters were 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. After amplification, the banding pattern of the randomly amplified DNA was fractionated by electrophoresis on 1.2% agarose gel and run at 65 V in 1X TBE buffer containing 0.5 µg ethidium bromide mL⁻¹. Gels were imaged with the gel documentation system (UVP, USA). All experiments were repeated three times under all conditions to test day-to-day reproducibility.

RESULTS

Figures 2 and 3 show RAPD patterns obtained with each primer (p1-p10) using different thermal block-based cyclers (1A, 1B and 2) on first, second and third day (Fig. 2A, 3A; 2B, 3B; 2C and 3C, respectively). The

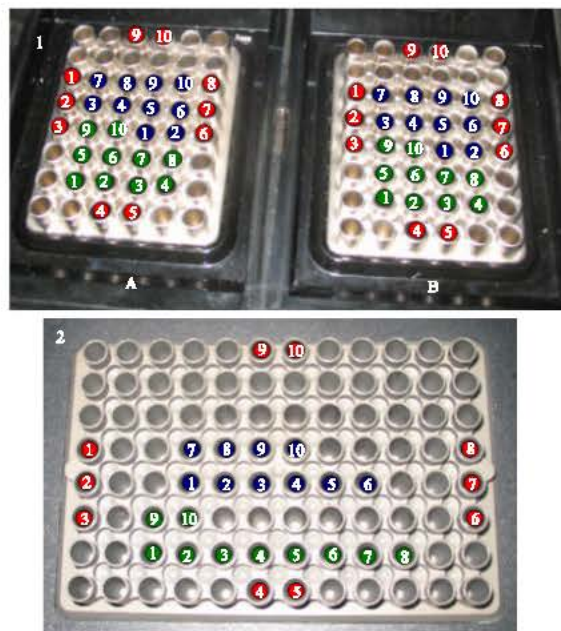


Fig. 1: MJ Research PTC thermal cycler: 1) MJR PTC-200 with a dual 48-well block (1A and 1B) and 2) MJR PTC-100 is a 96-well block, showing samples' places with different primers (1-10): Day one (green color), day two (blue color) and day three (red color)

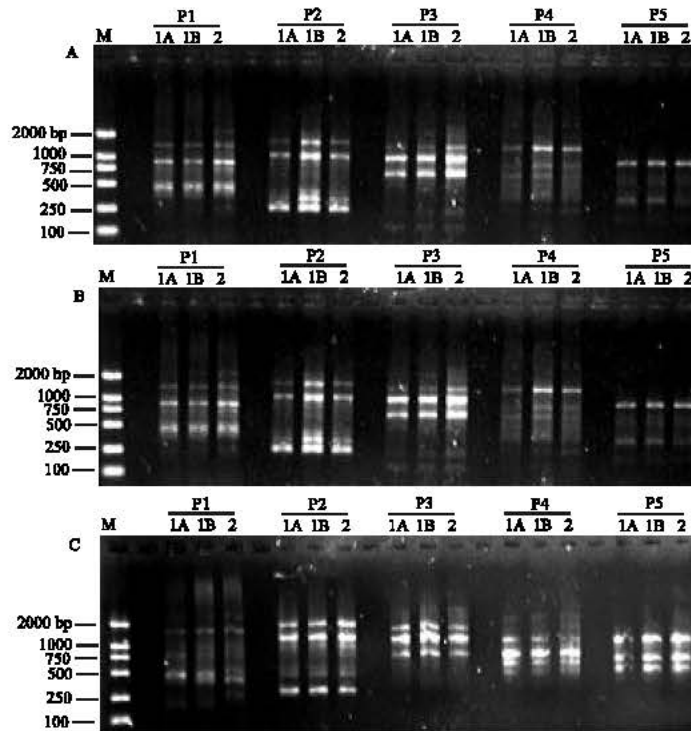


Fig. 2: RAPD patterns profile with primers (P1-P5), A) day one, B) day two and C) day three: Lane M: DNA molecular marker (DL 2 Kb). Lanes 1A, 1B and 2: Amplified by using MJR PTC-200 with a dual block and MJR PTC-100 single block with each primer, respectively

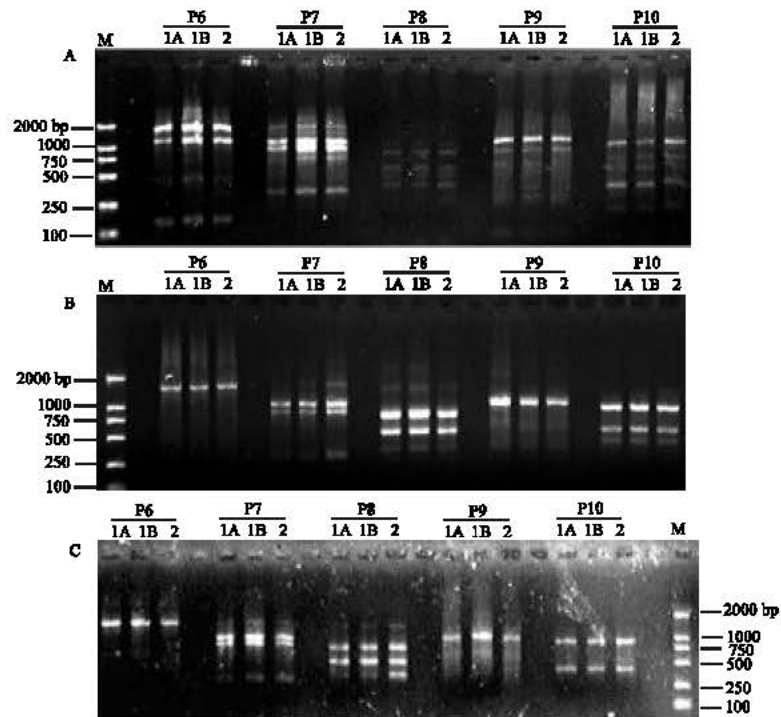


Fig. 3: RAPD patterns profile with primers (P6-P10), A) day one, B) day two and C) day three: Lane M: DNA molecular marker (DL 2 Kb). Lanes 1A, 1B and 2: Amplified by using MJR PTC-200 with a dual block and MJR PTC-100 single block with each primer, respectively

comparisons of RAPD fingerprints with each primer in the same day by using different thermal cyclers gave evidence for excellent reproducibility (Fig. 2 and 3). When primers p1, p4 and p6-10 used, the day-to-day reproducibility was poor and RAPD fingerprint variations occurred (appearance or disappearance of bands). On the contrary, 3 primers (p2, p3 and p5) gave evidence for good day-to-day reproducibility.

DISCUSSION

To ensure that the amplified DNA bands originated from genomic DNA and not primer artifacts, negative control (without template DNA) was carried out for each reaction. It has been observed that amplification smear and/or pattern which was specific to the *Taq* DNA polymerase has been detected in the control tube (not shown). This result is in agreement with the findings of other scientists (Williams *et al.*, 1990; Meunier and Grimont, 1993). This natural contamination of *Taq* is especially evident when primers are universal (Bottger, 1990). However, contamination did not affect the resulting pattern, since none of the bands in RAPD pattern matched the bands in control tubes.

RAPD analysis was found subject to day-to-day and lab-to-lab variability and even to depend on the thermocycler used (Devos and Gale, 1992; Meunier and Grimont, 1993; Penner *et al.*, 1993). Reproducibility requires that rare amplification events be either completely avoided or freely accommodated. Rare annealing events occur even under stringent amplification conditions and usually involve primer-template mismatching early in the thermocycling reaction. It has been reported that once mismatched annealing occurs the resulting amplification products have annealing sites perfectly complementary to the primer. Artifactual non-genetic variation in RAPD analysis has been shown to be considerable in the absence of appropriate optimization of primer and template concentrations and annealing temperature (Ellsworth *et al.*, 1993; Muralidharan and Wakeland, 1993). Furthermore, RAPD inconsistencies probably arise from the use low primer-template ratios that can result in borderline experimental conditions ill-defined during optimization by the inadequate resolution of amplification products. The comparison of RAPD fingerprints between laboratories should be done with utmost care (Meunier and Grimont, 1993). On the other hand, the GC content of primers used in this study ranged from 50 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).

The RAPD method is reliable in strictly defined conditions and when used to answer limited problems such as typing of a collection of unknown isolates, which does not necessitate between-laboratory comparisons. It can be concluded that reproducible fingerprints can be produced from replicate DNA preparations by different operators in independent experiments over time and using thermal block-based cyclers. An exchange of RAPD data between laboratories should take into consideration the sources of variations and standardized methodology should be devised.

ACKNOWLEDGEMENTS

This study was supported by grant from the National Nature Science Foundation of China.

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