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

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**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* *tenebrionis* (tt4) and *B. thuringiensis* *israelensis* (1977)) 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 as 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 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-Zaeeem *et al.*, 2006; Salem *et al.*, 2005).

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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 (977)] 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

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotides sequences (5’-3’)</th>
<th>GC%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>GGT GCG GGA A</td>
<td>70.00</td>
<td>Nilsson et al. (1998)</td>
</tr>
<tr>
<td>P2</td>
<td>GTT TCG TCC C</td>
<td>60.00</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>GTA GAC CCG T</td>
<td>60.00</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>AAG ACG CAG T</td>
<td>60.00</td>
<td>Ready-To-Go</td>
</tr>
<tr>
<td>P5</td>
<td>AAC GCG CAA C</td>
<td>60.00</td>
<td>RAPD Analysis</td>
</tr>
<tr>
<td>P6</td>
<td>CCC GTC AGC A</td>
<td>70.00</td>
<td>Kit (27-9500-01), Amersham Biosciences</td>
</tr>
<tr>
<td>P7</td>
<td>CCG AGT CCA</td>
<td>66.70</td>
<td></td>
</tr>
<tr>
<td>P8</td>
<td>ACG GCC CCT</td>
<td>77.80</td>
<td></td>
</tr>
<tr>
<td>P9</td>
<td>CCG GCG GCG</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

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 977).
Table 2: Genetic similarity estimated for each primer among the five bacterial strains

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL21 × C1a</td>
<td>66.67</td>
<td>33.33</td>
<td>75.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>00.0</td>
<td>83.33</td>
<td>85.71</td>
<td>71.56</td>
</tr>
<tr>
<td>BL21 × 66</td>
<td>40.00</td>
<td>25.00</td>
<td>28.57</td>
<td>46.15</td>
<td>66.67</td>
<td>33.33</td>
<td>00.0</td>
<td>16.67</td>
<td>28.57</td>
<td>31.26</td>
</tr>
<tr>
<td>BL21 × t4</td>
<td>20.00</td>
<td>25.00</td>
<td>28.57</td>
<td>18.81</td>
<td>40.00</td>
<td>16.67</td>
<td>00.0</td>
<td>42.86</td>
<td>22.22</td>
<td>26.01</td>
</tr>
<tr>
<td>BL21 × i977</td>
<td>20.00</td>
<td>33.33</td>
<td>44.44</td>
<td>30.77</td>
<td>33.33</td>
<td>00.0</td>
<td>00.0</td>
<td>16.67</td>
<td>00.0</td>
<td>19.84</td>
</tr>
<tr>
<td>C1a × 66</td>
<td>33.33</td>
<td>33.33</td>
<td>57.14</td>
<td>46.15</td>
<td>66.67</td>
<td>33.33</td>
<td>00.0</td>
<td>16.66</td>
<td>35.88</td>
<td></td>
</tr>
<tr>
<td>C1a × t4</td>
<td>25.00</td>
<td>60.00</td>
<td>28.57</td>
<td>28.57</td>
<td>40.00</td>
<td>16.67</td>
<td>50.0</td>
<td>28.57</td>
<td>00.0</td>
<td>30.82</td>
</tr>
<tr>
<td>C1a × i977</td>
<td>25.00</td>
<td>85.71</td>
<td>22.22</td>
<td>28.57</td>
<td>33.33</td>
<td>60.0</td>
<td>33.33</td>
<td>16.67</td>
<td>00.0</td>
<td>27.20</td>
</tr>
<tr>
<td>66 × t4</td>
<td>00.00</td>
<td>60.00</td>
<td>33.33</td>
<td>54.54</td>
<td>57.14</td>
<td>83.33</td>
<td>33.33</td>
<td>71.42</td>
<td>66.67</td>
<td>51.08</td>
</tr>
<tr>
<td>66 × i977</td>
<td>00.00</td>
<td>28.57</td>
<td>50.00</td>
<td>54.54</td>
<td>50.00</td>
<td>54.54</td>
<td>00.0</td>
<td>50.00</td>
<td>66.67</td>
<td>39.37</td>
</tr>
<tr>
<td>t4 × i977</td>
<td>100.0</td>
<td>66.67</td>
<td>75.00</td>
<td>100.0</td>
<td>85.71</td>
<td>72.73</td>
<td>40.0</td>
<td>71.73</td>
<td>80.0</td>
<td>76.87</td>
</tr>
</tbody>
</table>

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, C1a, 66, t4 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, C1a, 66, t4 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, C1a). While, the highest similarity (100%) between the B. thuringiensis strains (t4 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; C1a; i977 and t4 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; Panchuk et al., 2002). We tested the ability of this method to differentiate between five bacterial strains from two different genera. To ensure that the amplified DNA bands originated from genomic DNA and not primer artifacts, negative control was carried out.
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).

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REFERENCES


