Genetic Diversity among Pseudomonas aeruginosa Isolated From Different Sources in Al-Diwaniyah City, Iraq Using RAPD-PCR Technique

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Abstract. Pseudomonas aeruginosa is an aerobic Gram-negative bacterium which has emerged as one of the most problematic nosocomial pathogens. To characterize P. aeruginosa strains that are widespread in patients in Al-Diwaniyah city, 30 clinical and environment samples were collected from general hospitals of Al-Diwaniyah city. Methods for isolation and identifying P. aeruginosa based upon culture methods coupled with biochemical tests, A total of 11 amplified DNA fragments ranging in size from 250 to 1 Kpb were observed using two random amplified polymorphic DNA (RAPD) primers (opX-13, op Y-04) where as 22 polymorphic amplified fragments were commonly detected among the 6 P. aeruginosa isolates and each of primer give different genetic profiles. The results shows the RAPD-PCR analysis is a good epidemiological screening method to detect genetic variation among pseudomonas aeruginosa isolates.

Keywords: pseudomonas aeruginosa, Nosocomial, RAPD, PCR, Al-Diwaniyah, Iraq

Introduction

Pseudomonas aeruginosa is gram negative, obligate an aerobic and non- sporulation, is ubiquities organisms widely spread in soil, water and living hosts and motile through polar flagellum [1]. P. aeruginosa is of a significant resistance to a wide range of drug types. In addition to that, it is capable of acquiring resistance by mutation processes and harboring integrons that have numerous genes of resistance like the ones that code for “metallo-β-lactamases” (MBL) which are capable of cleaving the most active anti-microbial agents against it (carbapenems) [2]. Several P. aeruginosa virulence factors which may contribute to pathogenesis during (i) respiratory tract’s initial colonization and (ii) persistence during chronic infection have been characterized. Pili, nonpilus adhesins, and bacterial motility have been shown to be important in respiratory tract’s initial colonization [3]. The source of P. aeruginosa in intensive care units (ICUs) may be endogenous or exogenous; isolates having certain geno-types are considered to be potentially endogenous whereas the ones that have identical geno-type with any of the patients or the environmental samples are considered to be
potentially exogenous [4]. The best control of *P. aeruginosa* outbreaks could need fast recognition and strain differentiating. Typically, it was typed based on its phenotypic properties but this approach could be lacking in discriminatory powers and stability [5][6]. Because of its speed and versatility PCR has become a very fast and reliable tool for molecular biology based diagnosing of different infectious diseases. An example of the PCR – based methods is the random amplified polymorphic DNA (RAPD). It was commonly utilized for epidemiological research and phylogenetic of many microorganisms [7][8][9].

**METHODOLOGY**

**Sampling**

Thirty samples were collected from clinical and environmental cases. Samples were taken from out and inpatients who admitted to Al-Diwaniyah General Teaching Hospital and Al-Diwaniyah Hospital for Maternity and Pediatric Hospital. Between January 2018 to June 2018.

**Genotypic identification**

**DNA Extraction**

DNA of *P. aeruginosa* isolates have been obtained and purified with the use of the Extraction and purification Kit from “Geneaid” firm (UK).

**Primers**

Two arbitrary or random primers (OPX-13 ,OPY-04) obtained from Bioneer, IDTDNA(USA). Bacterial isolates were tested for single primers for RAPD-PCR technique (table1).

| Primer | Sequence 5--------3 |
|--------|---------------------|
| OPX-13 | 5- ACG GGA GCA A-3 |
| OPY-04 | 5- GGC TGC AAT G-3 |

**RAPD-PCR amplification**

Final product of 30μl reaction volumes containing 10 ul of single primer ,12.5 ul of green master combine ,5 ul of Genomic deoxyribonucleic acid and also the reaction volume has been rounded to thirty ul with the addition of two.5 ul of nuclease free water Amplification has been dispensed in a very thermo-cycler (Eppendorf) programmed for three mins. At a temperature of 94 degrees Celsius; for 45cycles 1 min. at a temperature of 94 degrees Celsius, 1 min at a temperature of 40 degrees Celsius and 2 mins at a temperature of 72 degrees Celsius; and a final extension of 7 mins at a degree of 72 degrees Celsius. Amplification product were electrophoresed in one.8% agarose gels then visualised via staining with ethidium bromide. Standard molecular markers were conjointly enclosed in every electrophoresis run. Ultraviolet trans-illuminated gels have been captures as photographs [10].

**Phylogenetic Analysis:**

Locations of without ambiguity scorable RAPD bands have been remodeled to a matrix of binary characters (“1” for the existence of a band at a certain location and “0” for the
opposite). Phylogenetic tree has been generated via the “un-weighted pair-group method
arithmetic (UPGMA)” average cluster analyzing.

Results and Discussion

**RAPD analysis of *pseudomonas aeuroginosa***

Polymorphism assay for *pseudomonas aeuroginosa* isolates has been done with the use of two
primers. Random amplification of the DNA of *p. aeuroginosa* isolates reveals the efficacy of
these selected nucleotides sequences in determination the similarity or variations among all
isolates.

![Figure 1](image1.png)

**Figure 1.** RAPD–PCR using the primer OPX-13. [M Line (ladder), the isolates numbered
(1,2,3,4,5,6) were positive for OPX-13 primer]

![Figure 2](image2.png)

**Figure 2.** RAPD–PCR using the primer OPY-04. [M Line (ladder), the isolates numbered
(1,2,3,4,5,6) were positive for OPY-04 primer]
**Figure 3:** Dendrogram analysis that shows phylogenetic diversity of 6 *pseudomonas aeuroginosa* isolates which is characterized by RAPD markers

|     | Pseudo1 | Pseudo2 | Pseudo3 | Pseudo4 | Pseudo5 | Pseudo6 |
|-----|---------|---------|---------|---------|---------|---------|
| Pseudo1 | 0       | 0.286   | 0.286   | 0.286   | 0.750   | 0.875   |
| Pseudo2 | 0       | 0.250   | 0.250   | 0.800   | 0.900   |         |
| Pseudo3 | 0       | 0.000   | 0.667   | 0.778   |         |         |
| Pseudo4 | 0       | 0.667   | 0.778   |         |         |         |
| Pseudo5 | 0       | 0.200   |         |         |         |         |
| Pseudo6 |         | 0       |         |         |         |         |

Similarity Matrix computed with Pearson coefficient
Figure 4: Dendogram analysis that shows phylogenetic diversity of 6 *Pseudomonas aeruginosa* isolates characterized by markers of RAPD

|        | pseudo1 | pseudo2 | pseudo3 | pseudo4 | pseudo5 | pseudo6 |
|--------|---------|---------|---------|---------|---------|---------|
| pseudo1| 0       | 0.250   | 0.800   | 0.500   | 0.600   | 0.667   |
| pseudo2| 0       | 0.750   | 0.400   | 0.500   | 0.600   |         |
| pseudo3| 0       | 0.833   | 0.333   | 0.800   |         |         |
| pseudo4| 0       | 0.667   | 0.200   |         |         |         |
| pseudo5| 0       | 0.833   |         |         |         |         |
| pseudo6| 0       |         |         |         |         |         |

Similarity Matrix computed with Pearson coefficient
Figure 5: Dendrogram analysis that shows phylogenetic diversity of 6 *Pseudomonas aeuroginosa* isolates characterized by RAPD markers using OPX-13, OPY-04

**Genetic characterization of *P. aeuroginosa* isolates by RAPD analysis**

A total of 11 amplified DNA fragments ranging in size from 250 to 1 Kpb were observed using two random amplified polymorphic DNA (RAPD) primers (opX-13, opY-04) where as 22 polymorphic amplified fragments were commonly detected among the 9 *P. aeuroginosa* isolates and each of primer give different genetic profiles.

UPGMA analysis for the dendrogram made based on the RAPD data generated by primer OPX-13 were performed and shown in figure 3. Analysis showed that the 6 *P. aeuroginosa* strains were grouped into two clustered and three classes. The large class comprised the pseudo4, pseudo3; a second class included strain of the pseudo1; and the third remaining class corresponded to the pseudo 2, the second cluster comprised of 2 classes one class includes pseudo 5 and second class includes pseudo 6.

UPGMA analysis for the dendrogram made based on the RAPD data generated by primer OPY-04 were performed and shown in fig.4. Analysis showed that the 6 *P. aeuroginosa* strains were grouped into two clustered and three classes. The large class comprised the pseudo 4, pseudo 6; a second class included strain of the pseudo2 and pseudo 1; and the third remaining class corresponded to the pseudo 3 and pseudo 5.

Genetic finger-printing and phylogenetic diversity between completely varying *P. aeuroginosa* isolates have been found by changing RAPD information to a Jaccard matrix of similarity and analyzed by UPGMA to supply a phylogenetic tree. The obtained desoxyribonucleic acid band pattern is comparable to a barcode, permitting to identify everyone. For example, isolate Sa1 presents distinctive bands once its desoxyribonucleic acid amplified with the majority of the primers that have been tested (Figure 1).

RAPD was used successfully for *P. aeuroginosa* and is less costly and time consuming than other methods. The RAPD approach is rather simple and requires no preceding knowledge about the nucleotide sequence of target organism. In addition to that, it’s of a high speed...
quick and convenience, since a strain may be typed in 48 hours of the harvest of the cells from the agar environment. It’s of a high degree of sensitivity, needs minimal amount of template DNA [11][12]. Furthermore, RAPD-PCR is discriminatory because it analyzes the whole genome. However due to its sensitivity and reproducibility, can be affected by small variations in reaction mixture and temperature cycles [13][14]. Thus, care is needed to standardize the procedure if it is to be used for routine analysis. RAPD analysis would be the most appropriate choice for epidemiologic studies as it is not expensive, smaller amount of DNA are required to provide high amplicons which can be easily interpreted without the need of imaging software [15][16]. Positive (type strain template DNA) and negative (no template DNA) have to be included at all times. The failure of many primers in the amplification of DNA could be because of their need to certain requirements for amplifications according to PCR-reagents or temperature profile, due to the fact that each reaction parameter was identical for every primer [17]. In addition to that, variations of banding patterns are possible because of certain needs of a specific primer.

The G+C content of the primer could in later be interfering with the PCR yielding , the best DNA template concentricity varied according to the primer that has been utilized [18].

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