Detection of *lasB* Gene of *Pseudomonasaeruginosa* Causing Different Infection

Rana M. Abdullah Al-Shwaikh[1]* and Abbas Falih Al-Arnawtee
[1]Department of Biology, College of Education Ibn-Al Haitham, University of Baghdad.
*Corresponding Author: dr.rana_alshwaikh@yahoo.com

Abstract

The result obtained 75 isolate of *Pseudomonasaeruginosa* included: 28 isolates from otitis media, 23 isolates from burn infections, 10 isolates from wound infections, 8 isolates from urinary tract infections and 6 isolates from blood. The isolates were identified by culturing on MacConkey agar, Blood agar, Cetrimide agar, Pseudomonas agar and CHROMagar Orientation then identified by performing biochemical tests including (oxidase test and catalase test) and further identification by using a API20E system. The result obtained *Pseudomonasaeruginosa* that have *Las B* included : 23 isolates from otitis media 82.14%, 19 isolates from burn infections 82.60%,10 isolates from wound infections 100%, 5 isolates from urinary tract infections 62.5 % and 6 isolates from blood 100%.The result revealed that the *lasB gene* was present in 63 isolates 84% of *Pseudomonasaeruginosa*. The gel electrophoresis showed that the molecular weight of *alg D* gene was 300bp. [DOI: 10.22401/ANJS.00.2.07]

Conclusions: The results of the detection the virulence gene showed that *Pseudomonasaeruginosa* have *las B* gene84% that encoded elastase enzyme as the enzyme is highly efficient in protein analysis and the necrosis process.

Key words: *Pseudomonasaeruginosa*, *Las B*gene.

Introduction

P. aeruginosa produces a variety of enzymes such as Alkaline Protease and Elastase, Alkaline Protease combines with Elastase to analyze Collagen and Elastin [1]. When the enzyme secretion in large quantities, necrosis occurs in the injury zone Especially in the case of lung infections in patients with cystic fibrosis, which leads to the destruction of epithelial cells [2]and has the ability to analyze soft tissue and many molecular biologics effective such as Fibrinogen [3] and Components of the Complementary System [4]. Proteases Enzymes also have an important role in increasing the virulence of *P.aeruginosa* bacteria especially when their extracellular secretion is important in destroying host tissues [5].

Elastase is one of the main virulence factors of *P.aeruginosa* and is associated with severe tissue damage during the invasion of the tissue by the bacteria [6]. Its virulence lies in its ability to break Elastin, a protein that is an important component. Elastase has an important role in determining the virulence of *P.aeruginosa* during the time of infection [7]. Proteases have different molecular weights from 20-60 kDa, most of which are extracellular. *P.aeruginosa* produce many proteases, and these proteins was Protease IV have a molecular weight of 26 kDa. As well as the type alkaline protease, which is the type of metallic extrusion cellular [8]. As well as Las A protease, which is also extracellular, molecular weight (20) kDa and elastase with a molecular weight of 33 kDa [9].

The elastase enzyme is encoded by *las B* gene, as the enzyme Las B elastase is highly efficient in protein analysis and the necrosis processes [10]. The process controlling by *las B* gene is performed during the Quorum-Sensing Transcription process [11].
The aim of this study is to detect *Las B* virulence genes by using PCR techniques.

Materials and method

1. **Collection of bacteria**: Bacteria were collected from different infection cases including burin and wound infection, otitis media, urinary tract infection and blood samples.
2. **Identification of bacteria:** identification of the isolates by culturing on media culture including MacConkey agar, Blood agar, Cetrimide agar, Pseudomonas agar and CHROMagar Orientation then identified by using biochemical tests

including (oxidase and catalase test) and further identification by using a API20E system [12].

3. **DNA isolation:** DNA kit was used for extracting DNA of bacterial for isolates (Geneaid Biotech kit system, UK) according to the manufacturer's instructions.
4. **Detection of *lasB* gene:** To determine *las B* gene of *P.aeruginosa* isolates by using the primer *lasB* 300 bpF: (GGAATGAACGAAGCGTTCTC), R: (GGTCCAGTAGTAGCGGTTGG).

Detection of the gene *lasB* attended the solution concentration of 10 pekmole/µl (by taking 10 µl from Stock solution and addition of 90 µl of distilled water Alloaona) The Stock solution was saved under −20 °C. Polymerase chain PCR interactive and conditions were described below [13].

Step	***Program***	
1	Initial denaturation at 94ºC for 3 min 1 cycle	
2	30 cycles	
	A	Denaturation DNA template at 94 °C for 30 sec.
	B	Annealing at 55 ºC for 1 min
	C	Extension at 72 ºC for 1 min.
3	A final extension at 72 ºC for 5 min 1 cycle	

5. **Separation of DNA bands:** PCR products were separated on a 2% agarose with 5 µl Ethidium bromide Bio Basic INC (Canada), at 50 vol. for 2 hrs, using 100 bp Ladder. The DNA bands were visualized and photographed under UV light Optima (Japan) [14].

Results and discussion

A total of 75 isolates of *P.aeruginosa* were isolated including : 28 isolates from otitis media, 23 isolates from burn infections, 10 isolates from wound infections, 8 isolates from urinary tract infections and 6 isolates from blood. The isolates were identified by culturing on MacConkey agar, Blood agar, Cetrimide agar, Pseudomonas agar and CHROMagar Orientation then identified by performing biochemical tests including oxidase and catalase test and further identified by using a API20E system as shown in table (1).

Table (1)
Preliminary diagnostic test results for Pseudomonas aeruginosa.

No.	***Test***	***Result***
1	Gram stain	−ve Rods
2	MaConkey agar	non-Lactose fermented
3	Blood agar	β-haemolytic
4	Cetrimide agar	Yellow
6	Pseudomonas agar	Green or creamy green
7	CHROM agar Orientation	Green or creamy green
8	Catalase test	+
9	Oxidase test	+

Result revealed that the *lasB* gene was present in 63 isolates (84%) of *P.aeruginosa*. *Pseudomonasaeruginosa* have *LasB* included: 23 isolates from otitis media (**82.14%**), 19 isolates from burn infections (**82.60%**), 10 isolates from wound infections (100%), 5 isolates from urinary tract infections (**62.5%**) and 6 isolates from blood (100%) as shown in table (2). The gel electrophoresis showed that the molecular weight of *lasB* gene was 300 bp. (Figure1).

Table (2)
Number and percentage of lasB gene of P. aeruginosa.

No.	*Sources*	*No.*	*lasB(%)*
1	Otitis media	23	(82.14)
2	Burn	19	(82.60)
3	Wound	10	(100)
4	UTI	5	(62.)(
5	Blood	6	(100)
Total		63	(84)

This study was similar with this carried out by Wolska and Szweda (2009) [15] who showed 84.6% of the isolated *P. aeruginosa* have *lasB*, and agree with Shi *et al.* (2012) [16] who found that 80% of *P. aeruginosa* have *lasB*, Sabharwal*et al*. (2014) [17] found that 75% of *P. aeruginosa*isolates confer this gene.

The results showed *that* a high proportion of clinical isolates possessed the *lasB* gene because it encodes the production of the enzyme elastase [10], which is an important virulence factor that helps *P. aeruginosa* to break down Elastin protein. This protein is an important component for human blood vessels and responsible for its elasticity. Elastin is also a major constituent of lungs, responsible for the process of lung expansion and contraction. Therefore, the enzyme Elastase plays an important role in determining the virulence of *P.aeruginosa* during the infection [7].

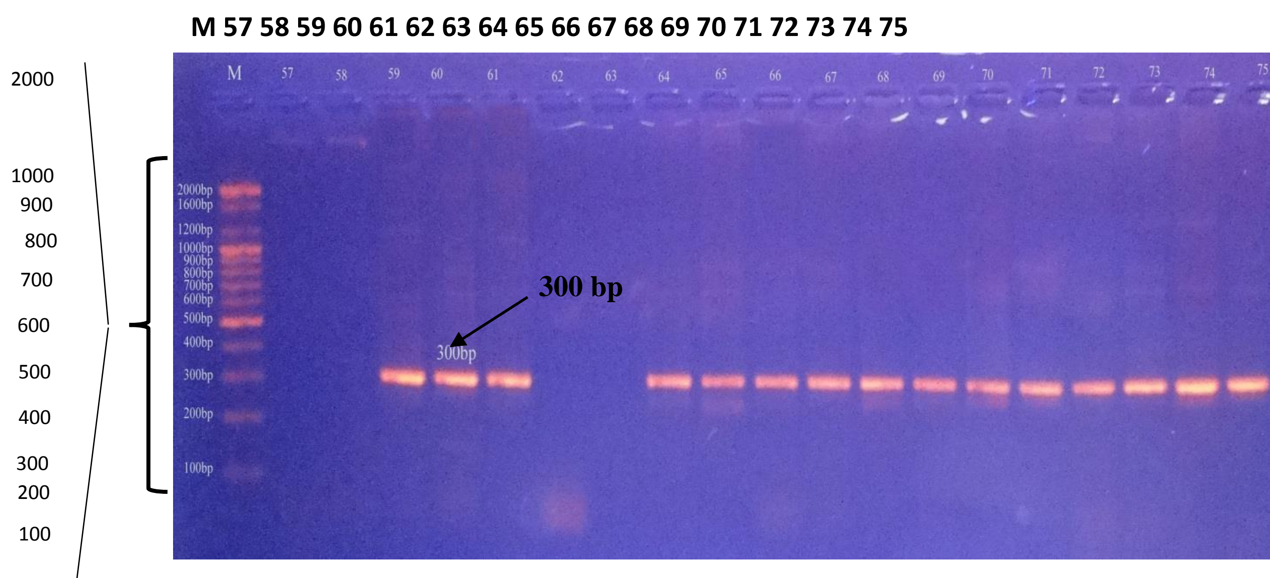

Figure (1) Agarose gel electrophoresis (2%) of PCR products of las B gene (300 bp). Line M: DNA marker (100 bp-2000 bp ladder, Promega, USA); Lanes 57-75 Pseudomonasaeruginosa PCR-positive isolates.

Conclusions:

The results of the detection the virulence gene showed that *Pseudomonasaeruginosa* have *lasB* gene (84%) that encoded elastase enzyme as the enzyme is highly efficient in protein analysis and the necrosis process.

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