Detection lipase gene of *Pseudomonas aeruginosa* from crude oil contaminated soil

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Abstract

The microbial lipases are industrially more substantial. The bacterial lipase enzymes can be extracellular and intracellular, and are extremely affected by bacterial nutrition and various physicochemical factors like temperature degree, pH, the sources of carbon and nitrogen, inorganic salts and agitation. The objective of current work is isolation and identify of *P. aeruginosa* from crude oil contaminated soil depending on PCR targeted lipA gene. 20g (15 samples) of crude oil contaminated soil were collected from north oil refineries/Kirkuk /Iraq. The isolates were identified according to microscopically diagnosis and colonial properties, biochemical tests, API-20E system with diagnosis by PCR technique based on gene lip A. PCR products by electrophoresis demonstrated only 8 isolates (53.33%) with positive results with lipA 558 from all isolates of *P. aeruginosa*.

Keywords: *P. aeruginosa*; lipase gene; polymerase chain reaction (PCR).

1. Introduction

Pseudomonads bacteria define as a large group of bacteria, which live originally in soil and the fresh water. *P. aeruginosa* is particularly widespread in the environments like soil, sewage, and related with certain plants [1-2]. *P. aeruginosa* (family: Pseudomonadaceae) is Gram negative, aerobic bacteria, rod-like shape, and its motile, Pseudomonadaceae comprise only genus Pseudomonas members that include eight groups and one of these groups is *P. aeruginosa* [3-5]. It succeed to grow not only in the typical atmospheres, but also in hypoxic (low oxygen levels) atmospheres, and has, thus, colonized many natural and artificial environments. It utilizes a broad range of organic substances for food [6-7]. In the contaminated soil, Pseudomonas spp. has specificity for degrading a various hydrocarbon compounds inclusive of biphenyl, petroleum/oil products and poly aromatic hydrocarbons [8-9]. Lipase enzymes are feature with hydrolases effect on the bonds of carboxylic ester which
found in acyl-glycerol to release the fatty acids and the glycerol [10-11]. Some lipase isoforms have been extracted and isolated from different bacterial cultures [12] and used in various applications in the food with dairy, and the detergent industries [13]. Therefore, the present study aims to detect the lipase gene in \textit{P. aeruginosa} isolated from soil.

2. Materials & methods

Sample collection

20g (15 samples) of crude oil contaminated soil were collected from north oil refineries/Kirkuk /Iraq utilizing sterile bottles to preservation and transport the samples to the laboratory.

Isolation and Identification

After collection of contaminated soil, then was cultured on cetrimide agar, blood agar and MacConkey for 24 h at 37 $^\circ$C for growth of colonies. The isolates were identified according to microscopically diagnosis and colonial properties, biochemical tests and API-20E system.

DNA Isolation

The genomic DNA of \textit{P. aeruginosa} was isolated according to method of [14]. The integrity and DNA purity was determined by using agarose gel electrophoresis.

PCR reaction

The PCR technique was done in reaction mixture (25µl) containing (7.5µl) of nuclease free water lipA 558, (12.5µl) of Green Master Mix and (3µl) of genomic DNA, 1 µl of each forward and reverse primers.

| Primer     | Sequence (5”-3””)                      | Product size bp |
|------------|----------------------------------------|-----------------|
| lipA 558   | FGGTCAACCTGCAGGGCCACAGCCACGGCG         | 558bp           |
|            | R GAGGCTGCAGACCTGTTACCTCGGTCCAGGTGG    |                 |
3. Results and discussion

Isolation and identification

The morphology, diameter and shapes of isolates on blood agar and MacConkey agar were determined. Also, the microscopic and biochemical examinations (table: 2) were done. The API 20E test (fig: 1) was done for identification.

**Table (2): biochemical tests of P. aeruginosa isolated**

| Biochemical tests | Catalase | Kliguler test | Motility | H₂S | Simmons citrate | V-P | M-R | Indol | EMB | Oxidase |
|-------------------|----------|---------------|----------|-----|----------------|-----|-----|-------|-----|---------|
| P. aeruginosa     | +        | -             | +        | -   | -              | +   | -   | -     | -   | +       |

**Figure (1): identification of bacteria by API 20E.**

In the current study, *P. aeruginosa* isolates were appear to be gram negative, oxidase positive, catalase positive, motile and producing bluish green coloration on cetrimide agar. The current findings agreeing with [15] who referred that *P. aeruginosa* had growth ability on specific media called cetrimide agar and its catalase positive results, oxidase positive. Also, results were agreeing with [16] who have described *P. aeruginosa* as Gram negative bacteria and the color of colonies on cetrimide agar is bluish green.
**P. aeruginosa lipase gene**

The primer of lipA 558 was used to amplification of P. aeruginosa lipase gene PCR technique (Figure 2).

![Agarose gel electrophoresis of PCR amplification products of P. aeruginosa lipA 558 on 1% gel of agarose (70 vol/90 min). Lanes 2-4: lipA 558 gene PCR product, lanes 1,5: ladder 100 bp.](image)

The PCR technique has been estimated for diagnosis of various bacterial types and other microorganisms in recent years [17-18] and it's especially beneficial for the raped identification and diagnosis of bacteria. PCR products by electrophoresis demonstrated only 8 isolates (53.33%) with positive results with lipA 558 from all isolates of P. aeruginosa and that may back to design of oligonucleotides (that used in present study) was not specific for P.aeruginosa lipA gene [19]. lipA gene was utilized to identified and diagnosis of P. aeruginosa, the lipase gene according to [20] might be utilized to classify and identified unknown types of Pseudomonas bacteria because the sequence homologies between genes of lipase enzymes are much less when comparing genes from distantly regarding Pseudomonads.

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