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Isolation and Identification of *lipA* Gene Producing *Pseudomonas aeruginosa* from Industrial Wastewater

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Abstract: Lipases are enzymes which have interesting in last few years for its widely applications in many industries like leather, detergents, cosmetic ,pharmaceuticals, biofuel, food, wastewater treatment etc. Many organisms produce lipase like animals, plants, fungi and bacteria. *Pseudomonas aeruginosa* lipases are very interesting to have some properties that are not common among lipases produced by other microorganisms, such as their thermoresistance and activity at alkaline pH ,that make it suitable to degrade oils in industrial wastewater. This study aimed to isolation and identify for *Pseudomonas aeruginosa* from industerual wastewater (vegetable oils) depending on the polymerase chain reaction (PCR) targeted *lipA* gene. Fifty samples were collected from oil rich industrial wastewater processed to routine biochemical tests to diagnosis *P. aeruginosa* and compare these results with diagnosis by PCR technique based on gene *lip A*, it was found that Thirty four of fifty samples (68%) positive results with *P. aeruginosa* . Tow *lipA* gene primers were used to detect for *P. aeruginosa* by the polymerase chain reaction Technique (PCR). Throw tow primers used, *lipA* 948 was the best and more specialized primer to isolation *P. aeruginosa* , (100%) positive result. While the second primer *lipA* 558, (66.66%) positive result.

Keywords: *lipA*, *P. aeruginosa*, PCR.

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Introduction:

Pseudomonas aeruginosa is gram negative rod, glucose non-fermenting, has the grape-like smell. It is a strict aerobe with a growth temperature range of 5-42°C. Most other pseudomonads will not grow at 42°C, have swarming motility by unipolar flagella(1,2). *P. aeruginosa* adapt easily to a large different of natural ecosystems ,it have the ability to survive on minimal nutritional requirements and to tolerate a variety of physical condition(3).

Lipid contamination caused many problems such as decreasing oxygen transfer rate, coating animals, plants and polluting water(4,5).

lipids require lipolytic enzymes during their metabolism. Lipolytic enzymes catalyze the turnover of these water-insoluble compounds(6). Lipases have been produced in many species of animal, plant and microorganism, microbial lipases are the most interisting for the industrial applications due to the useful properties related to their stability as organic solvent-tolerant and thermostable enzymes(7-9).

The degradation of lipid begins with bacteria usually secreting extracellular lipase, that broken down the ester bonds into glycerol and fatty acids(10,11). Lipases encoded by *lipA* gene is the key enzyme to degrade lipid(12).

LipA gene in *P. aeruginosa*, Nucleotide sequence analysis revealed a gene of 936 bp. codes for 311 amino acids(13). It will establish in different wastes treatment processes such as oil waste bioremediation(14).

Pseudomonas lipases display special biochemical properties not common among the lipase produce by other microorganisms, e.g., their thermorestance and activity at alkaline PH (15,16). The lipase *lipA* from *P. aeruginosa* binds to the extracellular polysaccharide. This binding localizes the enzyme near the cell surface and enhances the fairly stable of the enzyme towards heat(17,18).

Lipase is very important in degrade and dissolve lipids in the biological treatment of oil rich wastewaters, accelerating the process and improving time efficiency. The treatment of effluents from fats is a new and favourable application for lipases, because their ability to hydrolysis the triglycerides to free fatty acids and glycerol(19,20). The current study was aimed of isolation and identification of *lipA* gene producing *pseudomonas aeruginosa* from industrial wastewater.

Materials and Methods:

Sampling:

Oil rich industrial wastewater samples were collected from the factories of the general company of vegetable oils, from different departments of AL Rasheed ALameen factory, While the Sewage samples were collecting from sewer service Baghdad /Alrustumaiya.

Labrotary identification of isolates:

The samples were collected from both the general company of vegetable oils and from sewer service Baghdad were proessed to routine tests, include the morphological, bacteriological and biochemical tests which include grow on Cetrimide agar as a selective medium, MacConkey agar , growing at 42°C,gram staining, oxidase and catalase test. Triple Sugar Iron (TSI) test (glucose, lactose or Sucrose fermentation and H_2S production), urease test and IMViC tests indol, methyl red (MR), voges-Proskauer (VP) and Simmon's citrate agar) (21-24).

DNA extraction:

Over night bacterial cultures were inculated nutrient broth culture and incubated for 24 h at 37°C , one ml of the cultures were transferred to 1.5 ml microcentrifuge tube, centrifuged for 1 minute at 14-16000x g, then supernatant was discarded. The bacterial genomic DNA extract was done according to manfcture protocol by using Reagent Genomic DNA Kit (Geneaid -Thailand).

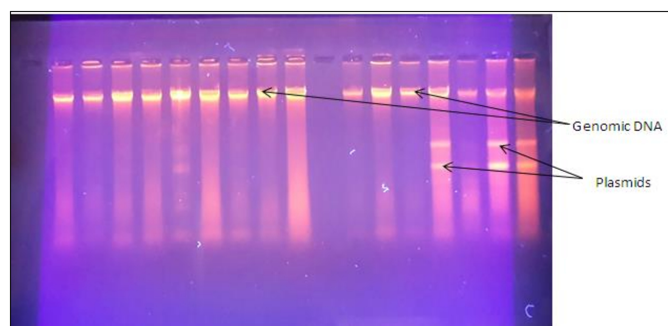

Figure (1): Electrophoresis of the extracted DNA on 1% agarose (70 vol/ 90 min) to check purity and integrity.

PCR reaction mixture set up:

The PCR was performed in 25 µl reaction maxture cntaining 12.5 µl of Green Master Mix (1X) (promega), 3 µl of genomic DNA of bacteria , 1 µl of each forward and reverse primers and 7.5 µl of nucleases free water *lipA* 558.

Table (1): Primer used in present study.

Primer	Sequence (5''-3'')	Reference	Product sizebp
*lip*A 948	F GGATCCATGAAGAAGAAGTCTCTGCT R AAGCTTCTACAGGCTGGCGTTCTT	Wu *et al.*, (12)	948bp
*lip*A 558	F GGTCAACCTGCAGGGCCACAGCCACGGCG R GAGGCTGCAGACCTGGTTCACCTCGTCCAGGTGG	Martinez *et al.*, (14)	558bp

The amplification condition of PCR reaction was optimized with following parameters: for universal primer *lipA* 558, an initial denaturation step at 95°C for 2 min. ,a denaturation step at 95°C for 45 sec.,annealing at 55°C for 45 sec. , extention at 72°C for 1.5 min. and final extention step at 72°C for 5 min. 25 serial cycles of reactin was performed.

For second primer *lipA* 948, an initial denaturation step at 94°C for 5 min. ,a denaturation step at 94°C for 1 min.,annealing at 60°C for 45 sec. ,extention at 72°C for 1 min. and final extention step at 72°C for 10 min. 30 serial cycles of reactin.

Results and Discussion:

Thirty four of fifty samples (68%) positive results for the routine tests that were used to detect the presence of *P. aeruginosa*.

The limitations of many traditional techniques particularly low specificity and long turnaround time(20), results. Polymerase chain reaction (PCR) is a better alternative than conventional method techniques. It is allow to give more rapid and accurate identification of the various scientific fields in a time substantially shorter and costly than traditional methods(25-27).

Amplification of lipase gene in *P. aeruginosa* by *lipA* 558:

Lipase gene of *P. aeruginosa* amplification with *lipA* 558 primer by PCR technique (Figure 2).

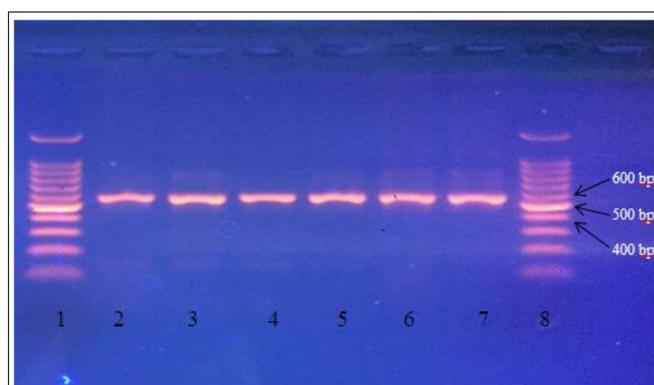

Figure (2): Agarose gel electrophoresis of PCR amplification products of *P. aeruginosa lipA* 558 on 1% gel of agarose (70 vol / 90 min). Lanes 2-7 : *lipA* 558 gene PCR product, lanes 1,8 : ladder 100 bp.

The examination of PCR products by electrophoresis, showed only 19 isolates (66.66%) gave positive results with *lipA* 558 from the total isolates number (34) and that may be due to the design of this oligonucleotides was not specific for *lipA* of *P.aeruginosa*, it was based on highly preserved region of 12 bacterial *lipA*-homologous genes. These bacteria are *P. aeruginosa, P. fragi, Burkholderia glumae, Burkholderia cepacia* and several *Pseudomonas* sp. Strains(15).

Jaric *et al*., (28) noted that most of the methods require an efficient PCR whose forward and reverse primers bind well to the same, large number of identifiable species, and produce amplicons that are unique , for this reason many researchs used universal primers designed were not as efficient and fail to bind to recently cataloged species.

Molecular identification of *P. aeruginosa* by *lipA* 948:

In this step of study lipase gene amplification with *lipA* 948 primer by PCR technique.

The examination of PCR amplification was appeared that all 34 isolates give positive result (100%) in identification of *P. aeruginosa*. This PCR primer for the lipase gene was designed based on the full length DNA sequences for *P. aeruginosa* lipase genes in NCBI GenBank. This primer was used in detection of *lipA* gene, isolated and cloned with another genes to enhance the production of the active lipase *lipA*(13). So, *lipA* 948 primer was more specific in detection of *lipA* gene in *P.aeruginosa* than *lipA* 558 (Figure 3).

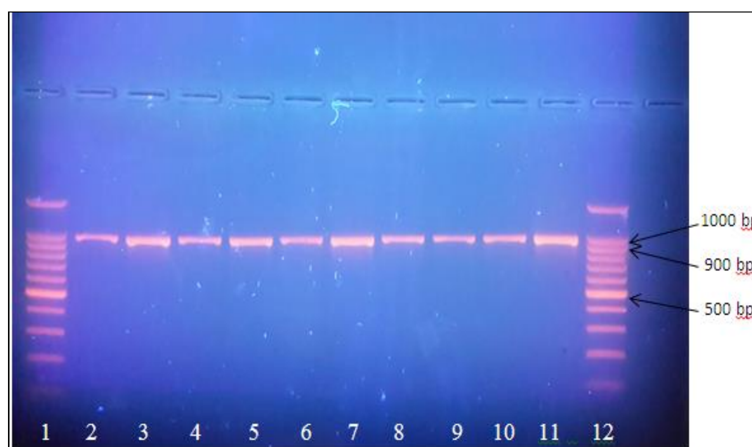

Figure (3): Agarose gel electrophoresis of PCR amplification products of *P. aeruginosa lipA* 948 on 1% gel of agarose (70 vol / 90 min). Lanes 2-11 : *lipA* gene PCR product, lanes: 1,12 ladder 100 bp.

Also *lipA* gene was used to identified *P. aeruginosa*, the lipase gene and it' s protein according to Wohlfarth *et al*., (28) might be used to classify unknown species of *Pseudomonas* because the sequence homologies between lipase genes are much less when comparing genes from distantly related *Pseudomonads*. For example, *lipA* of *Pseudomonas sp.* 42A2 showed highly homologous to those of *P. aeruginosa* PAO1(30). The lipases of *P. auruginosa* PAO1, *P. alcaligenes* DSM 50342 and

Pseudomonas sp. were showed a high degree of homology at the amino acid (89 %) as well as the nucleotide level. More distantly related strains showed lower homology at the amino acid level (63% with *P. cepacia)* and less when comparing nucleotide sequences. Vasil *et al* (31) obtained similar results when studying various strains of *P. aeruginosa* using the exotoxin A gene as a probe.

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