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# Isolation and Identification of the Dominant Bacteria From the Soil Contaminated with Hydrocarbon Waste

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**ABSTRACT**

The aim of this research to isolate and determine the most dominant bacteria in soil contaminated by hydrocarbons, due to this type of bacteria plays an effective role in bioremediation of hydrocarbon pollutants in the soil, thus reducing the danger of this type of pollutants on the environment. Commercial, industrial, and military activity, largely in the 19th and 20th centuries, have led to environmental pollution that can threaten human health and ecosystem function are the major sources of energy for industry and daily life that cause environmental contamination during various stages of production, transportation, refining and use. Four samples of contaminated soil were collected from Al-Amriya Gas Filling Factory in November, 2023 sampling of depth (0-15 cm) because at this depth, bacteria are active to provide the appropriate conditions for their growth from temperature, humidity, acidity pH and nutrients including hydrocarbon pollutants, then all samples transported to the laboratory. Results revealed that dominant isolates are *Kocuria rosea*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These isolates identified depending on morphological cultural, gram stain, microscopic features, biochemical tests, and VITEK2 compact.

## 1. Introduction

Contamination of soils by petroleum hydrocarbons causes drastic changes in microbiological, chemical, and physical properties of soil. Crude oil and natural gas are the two commonest petroleum hydrocarbon contaminants of soils [1]. Due to the dynamic increase in industrialization, urbanization, and the increasing demand for energy, pollution with persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), poses a serious threat to all forms of aquatic and terrestrial life

The improper disposal and management of LPG residues pose significant risks to environmental health and ecosystem integrity [2]. Soil, as a fundamental component of terrestrial ecosystems, plays a crucial role in supporting life by providing a habitat for diverse organisms, facilitating nutrient cycling, regulating water flow, and maintaining overall ecosystem stability [3]. However, when exposed to contaminants such as LPG waste, soil ecosystems face disruption and degradation, leading to adverse consequences for both environmental sustainability and human well-being [4].

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The contamination of soil by LPG waste arises from various sources, including accidental spills during transportation and storage, leakage from storage tanks or pipelines, and improper disposal practices [5]. These contaminants consist of a complex mixture of hydrocarbons, including volatile organic compounds (VOCs) and other toxic substances, which can persist in the environment for extended periods, exerting detrimental effects on soil quality and ecosystem functioning [6-8].

## 2. Materials and Methods

### *2.1 Sampling Site*

Al-Amriya gas filling factory belongs to general company for gas filling and services/Ministry of oil located in Baghdad city. 33°15'56"N 44°19'70"E figure (3-1). This factory was chosen in this study due to the abundance of hydrocarbon pollutants that are released from it into the soil.

**Figure 1.** Al Amriya gas filling factory

In 1958, began utilization of gas filling cylinders in Iraq, while in 1970 work hand full of liquid petroleum gas in Al-Taji with a production capacity of 8,000 standard cylinders in the day (GFC, 2016). The company has 53 government gas filling plants that it manages technically and administratively, and 237 private gas filling plants that it supervises. And 23 workshops for adding liquid gas systems to cars, and 47 stations and outlets for filling liquid gas for cars.

Gas filling company GFC administrates 51 governmental refineries and about 197 personal refineries that spread over all provinces, example for these gas refineries, Al Amriya for gas filling which the samples were collected and studied.

### *2.2 Collection of samples of polluted soils*

Four samples of contaminated soil were collected from Al-Amriya Gas Filling Factory in November, 2023 sampling of depth (0-15 cm) using a stainless hand trowel, each sample of soils was collected in plastic sterile bags, then all samples transported to the laboratory and stored at 4°C for 24 hours, after that using in the isolation of hydrocarbon degrading

bacteria conducted in Al-Taqadum laboratory in Baghdad figure 2.

**Figure 2.** Samples of contaminated SOILS

*2.3 Culture media*

*2.3.1 MacConkey agar*

MacConkey agar was prepared by dissolving 49.53g of dehydrated medium in 1000 ml of distilled water. It was boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. This medium used for differentiation between lactose fermenting and lactose non-fermenting bacteria.

*2.3.2 Nutrient agar*

Nutrient agar solution was prepared by dissolving 28g in 1000ml of distilled water. It was boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 14 atm pressures and 121°C for 15 min. This medium used for the growth bacterial isolate.

*2.3.3 Bacterial Stain: Gram stains*

*2.4 Isolation of bacteria*

One gram of each soil sample was added to 9ml of saline and shacked to homogenize, and then serial dilutions ($10^{-1}$ to $10^{-5}$) were prepared for each sample, 0.1ml of each dilution were added to plates and then nutrient agar medium added and incubated at 37Co for 24 hour, then bacterial colonies that different in morphological characteristics were purified by sub culturing on nutrient agar medium until pure culture was obtained and stored in refrigerator at 4Cº [13].

*2.4.1 Identification of bacterial isolates*

Most efficient bacterial isolates were identified according to the gram stain reaction, arrangement, and morphology as described in Bergey's manual of determinative bacteriology.

*2.4.2 Identification of bacteria using VITEK 2 device*

There are 47 biochemical tests and one negative control well. Final identification results are available in approximately 10 hours or less, this device contains 64 biochemical tests. The gram negative card is based on establish biochemical methods and newly developed substrates measurement carbon source utilization, enzymatic activities, and resistance figure 3 and 4.

## 3. Results and discussions

*3.1 Screening of bacterial isolates*

Several bacterial isolates were obtained from soil samples. The most dominant bacterial isolates were screened to select the most efficient isolates. The results showed that three out of bacterial isolates were more efficient in biodegradation that present in contaminated soil. Through the results obtained from soil samples collected from the gas filling plant in

Amriya, it was found that there are three samples that are the most dominant, and they are as follows: *Pseudomonas aeruginosa*, *Kocuria rosea*, and *Escherichia coli*, and according to studies the most efficient isolates in bioremediation of hydrocarbons are *Pseudomonas aeruginosa* and *Kocuria rosea* figure 1 and table 1.

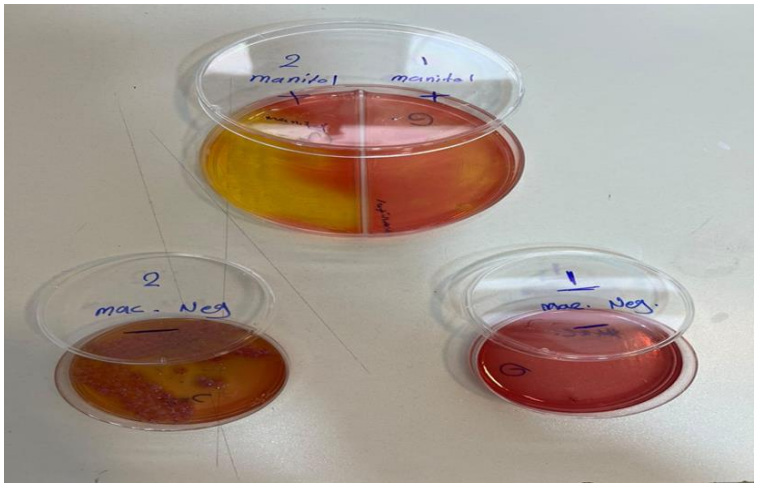


**Figure 3.** Isolates of bacteria

Bacteria *P. aeruginosa*, as well as many other *Pseudomonas*, can degrade contaminants in environment that are present in the soils and underground, and in surface water such as hydrocarbons by-products of petroleum industries thus, *P. aeruginosa* can be used in control of pollution. As well as *Kocuria rosea* it has great effectiveness in biodegradation of hydrocarbon pollutants because it contains genes that encode enzymes capable of degrade these pollutants with high efficiency. Therefore have unique ability to use crude oil and polyaromatic hydrocarbons (PAHs) as sole source of carbon and energy in growth (15,16). As for the reason for the presence of *Escherichia coli* bacteria in soil samples, it may be due to the presence of soil pollution with sewage or animal waste.

### *3.2 Identification of bacterial isolates by VITEK 2 compact device*

The bacterial isolates were identified by using VITEK 2 compact device.

The results were found in figures (4-2) and (4-3).

VITEK 2 is an automatic system for the identification and susceptibility testing of the most clinically and environmental important bacteria.

VITEK 2 compact device system was used in this study due to it gives several advantages compared with routine tests for identification of bacteria isolated from environmental samples; also it provides rapid identification, a high automation level, a simple methodology, and taxonomically updated databases.

**Table 1:** Bacteria Identified by VITEK 2 compact device

| Isolate No. | Result |
|---|---|
| **1** | *Pseudomonas aeruginosa* |
| **2** | *Kocuria rosea* |
| **3** | *Escherichia coli* |

*P. aeruginosa*, as well as *Kocuria rosea*, can degrade contaminants in environment that are present in the soils and underground, and in surface water such as hydrocarbons by-products of petroleum industries thus, *P. aeruginosa* can be used in control of pollution. The bacterium is ubiquitous in water, soil, and on surfaces in contact with soil or water, in nature *Pseudomonas* bacterium might be found in a biofilm, attached to some surface or substrate, or in form of a planktonic, as a unicellular organism. *P. aeruginosa* has very simple requirements for nutrition. Optimum temperature for Its growth is 30C$^{o}$, and it is able to grow at temperatures as high as 42$^{o}$C.

bioMérieux Customer: Microbiology Chart Report Printed December 12, 2023 12:37:16 PM CST

Patient Name: Dr nahla student, gn Patient ID: 182
Location: Physician:
Lab ID: 182 Isolate Number: 1

Organism Quantity:
**Selected Organism : Pseudomonas aeruginosa**

**Source:** **Collected:**

| **Comments:** | |
|---|---|

| **Identification Information** | **Analysis Time:** | 4.87 hours | **Status:** | Final |
|---|---|---|---|---|
| **Selected Organism** | 93% Probability | **Pseudomonas aeruginosa** | | |
| | **Bionumber:** | 0043453043504250 | | |
| **ID Analysis Messages** | | | | |

| **Biochemical Details** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | lARL | - | 7 | dCEL | - | 9 | BGAL | - |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | + | 13 | dGLU | + | 14 | GGT | + | 15 | OFF | - |
| 17 | BGLU | (-) | 18 | dMAL | - | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | - | 22 | BAlap | + |
| 23 | ProA | + | 26 | LIP | + | 27 | PLE | - | 29 | TyrA | - | 31 | URE | - | 32 | dSOR | - |
| 33 | SAC | - | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | + | 37 | MNT | + | 39 | 5KG | - |
| 40 | lLATk | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - |
| 46 | GlyA | - | 47 | ODC | - | 48 | LDC | + | 53 | lHISa | - | 56 | CMT | + | 57 | BGUR | - |
| 58 | O129R | + | 59 | GGAA | - | 61 | lMLTa | + | 62 | ELLM | - | 64 | lLATa | - | | | |

**Figure 4.** *Pseudomonas aeruginosa* identification via VITEK 2 compact device

bioMérieux Customer: Microbiology Chart Report Printed December 12, 2023 12:37:17 PM CST

Patient Name: Dr.nahla student, 1gp Patient ID: 184
Location: Physician:
Lab ID: 184 Isolate Number: 1

Organism Quantity:
**Selected Organism : Kocuria rosea**

**Source:** **Collected:**

| **Comments:** | |
|---|---|

| **Identification Information** | **Analysis Time:** | 7.80 hours | **Status:** | Final |
|---|---|---|---|---|
| **Selected Organism** | **Bionumber:** | **Kocuria rosea** 000010300020000 | | |
| **ID Analysis Messages** | | | | |

| **Biochemical Details** | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | AMY | - | 4 | PIPLC | - | 5 | dXYL | - | 8 | ADH1 | - | 9 | BGAL | - | 11 | AGLU | - |
| 13 | APPA | - | 14 | CDEX | - | 15 | AspA | - | 16 | BGAR | - | 17 | AMAN | - | 19 | PHOS | - |
| 20 | LeuA | + | 23 | ProA | - | 24 | BGURr | - | 25 | AGAL | - | 26 | PyrA | (-) | 27 | BGUR | - |
| 28 | AlaA | + | 29 | TyrA | + | 30 | dSOR | - | 31 | URE | - | 32 | POLYB | - | 37 | dGAL | - |
| 38 | dRIB | - | 39 | lLATk | - | 42 | LAC | - | 44 | NAG | - | 45 | dMAL | - | 46 | BACI | - |
| 47 | NOVO | - | 50 | NC6.5 | + | 52 | dMAN | - | 53 | dMNE | - | 54 | MBdG | - | 56 | PUL | - |
| 57 | dRAF | - | 58 | O129R | - | 59 | SAL | - | 60 | SAC | - | 62 | dTRE | - | 63 | ADH2s | - |
| 64 | OPTO | - | | | | | | | | | | | | | | | |

**Figure 5.** *Kocuria rosea* identification via VITEK 2 compact device

## 4. Conclusion

High level of pollution of soil, and water result from gas filling refineries GFR due to absence of moderns techniques in liquid petroleum gas LPG refineries for discharging wastes. Gram negative bacteria were dominant in soil contaminated with hydrocarbons because have enzymes help in consuming these compounds [9,10].

*Pseudomonas aeruginosa* and *Kocuria rosea* have high ability to degrade hydrocarbons; hence, it can be very useful for environmental protection from hydrocarbons wastes, as well as increase of growth of bacteria with increases of pollutant concentration but at an expended, after that any increase of pollutant concentration become toxic to the bacteria [11,12].

Regenerating bacteria via using cloning and recombinant DNA technologies to produce bacteria have higher ability to degrade hydrocarbons and strategically planning in construction of petroleum industry and gas filling refineries to avoid environmental problems as well as modern techniques must be used in discharging of wastes in gas filling refineries to reduce the pollution of soil [13]. The ability of isolated bacteria to degrade hydrocarbons in contaminated soil suggested that they could be used for the treatment of other oil, hydrocarbons and gas filling refinery wastes in soil and groundwater. Additional works will be suggested in our future research for the application of bioremediation using isolated strains [14].

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