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Isolation and Characterization of Some Hydrocarbons-Degrading Microorganisms from Oil-Contaminated Soil Samples from Kafr El-Sheikh and the Gulf of Suez Governorates, Egypt

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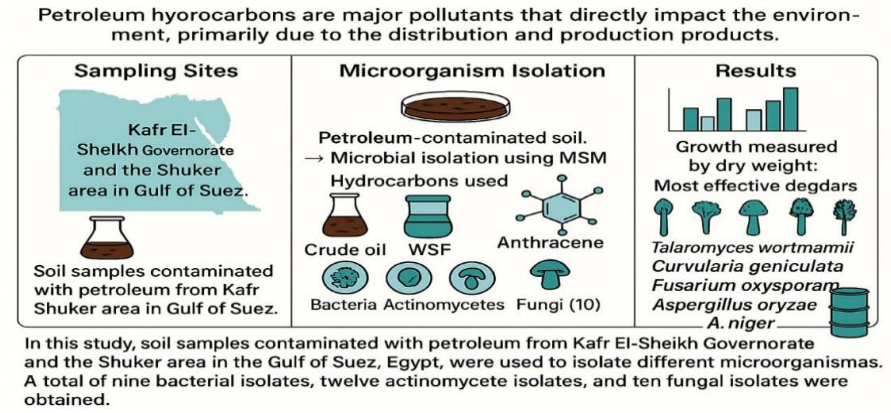
ABSTRACT

Petroleum hydrocarbons are major pollutants that directly impact environment, primarily due to the distribution and production of petroleum products. In this study, soil samples contaminated with petroleum from Kafr El-Sheikh Governorate and Shuker area in the Gulf of Suez, Egypt, were used to isolate different microorganisms. A total of nine bacterial isolates, twelve actinomycete isolates, and ten fungal isolates were obtained from these soil samples. The isolation was performed using mineral salt media containing crude oil, water-soluble fractions (WSF), or anthracene as the sole carbon source, with each substrate applied separately. Microbial growth was assessed by measuring dry weight. The results indicated that fungi, specifically *Talaromyces wortmannii, Curvularia geniculata, Fusarium oxysporum, Aspergillus oryzae, and A. niger*, were the most effective in degrading crude oil, WSF, and anthracene. Crude oil is the best hydrocarbon used for growth.

Graphical abstract



1. Introduction

The term petroleum has been used as a synonym for crude oil, which contains various hydrocarbon compounds. Petroleum contains complex compounds, including cycloalkanes, linear alkanes, polycyclic aromatic hydrocarbons (PAHs), resins, and asphaltenes, which are mostly carcinogenic and teratogenic compounds [1]. These pollutants leaked into the environment, causing serious damage to all living entities, and the refractory substances will enter the living animal body through the food chain, which seriously threatens human health and harms the ecosystems [2].

Hydrocarbon pollutants are persistent organic contaminants that cause long-lasting and harmful effects on ecosystems through biomagnification [3]. Their widespread release from sources like oil spills, leakage from tanks, and abandoned refinery sites contaminates soil, groundwater, and oceans [4-7]. Many petroleum constituents, such as BTEX (benzene, toluene, ethylbenzene and xylene) compounds, are known for their toxicity and volatility, making them significant environmental pollutants, especially in groundwater and air contamination scenarios. Benzene, in particular, is a recognized carcinogen, and PAHs (polycyclic aromatic hydrocarbons) are highly toxic and resistant to degradation, posing health risks due to their carcinogenic, hemotoxic, and teratogenic properties [8-10]. These pollutants impact living organisms by causing respiratory issues, metabolic disturbances, developmental abnormalities, and mortality, both in the short and long term [11-15]. They can lead to ecosystem shifts, affect species populations, and pose

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significant health hazards to humans, especially through volatile compounds capable of inducing tumors [16, 9].

A wide variety of microorganisms, including bacteria, fungi, and algae, are capable of degrading petroleum hydrocarbons in contaminated soil. These microorganisms play a crucial role in biogeochemical cycles and are key drivers in the breakdown of many petroleum hydrocarbons [17]. Among them, fungi offer notable advantages over other microbes in biodegradation because they can grow on a diverse range of substrates. Due to their enzymes, fungi can break down various environmental contaminants and utilize them as growth substrates [18]. Moreover, fungi possess the ability to chemically or physically mineralize pollutants through mechanisms such as oxidation-reduction reactions, binding, volatilization, immobilization, and chemical modification [19]. Through these diverse processes, microorganisms can effectively restore contaminated environments [20].

This study aims to isolate microorganisms capable of growing on different hydrocarbons and determine the most potent ones.

2. Materials and methods

2.1. Sampling

Two oil-contaminated soil samples from different localities in Egypt were used. One sample was obtained from DISUCO Company, Kafr El-Sheikh Governorate (No.1), Egypt (31° 12' 30.5"N) latitudes and (30°45'58.6"E) longitudes. The other sample was collected from the Shuker area, Gulf of Suez (No.2), Egypt (29° 53' 55" N) latitudes and (32° 32' 07" E) longitudes. The samples were collected from 15-20 cm from the soil surface and saved in sealed plastic bags, then stored until used. Analysis of soil samples was carried out at Desert Research Center (Central laboratory), El-Materia, Cairo, Egypt.

2.2. Determination of crude oil percentages in soil samples [21]

Oil content in soil samples was determined using two solvents, hexane and toluene, and applied at both room temperature and 70°C using a shaking water bath. For each sample, five grams of soil were placed into 250 ml conical flasks, along with 50 ml of the selected solvent. The flasks were then shaken thoroughly for 40 minutes on a rotary shaker set at 200 rpm. After shaking, the samples were centrifuged at 4000 rpm for 10 minutes. The solvent phase was collected in glass containers and dried at room temperature until reaching a constant weight. Each treatment was performed in triplicate.

2.3 Preparation of water-soluble fraction (WSF) [22]

A sample of crude oil (500 ml) was slowly mixed with water (500 ml); the crude oil-water mixture was stirred slowly for 24 hours with a magnetic shaker. The mixture was made to stand for 3 hours before it was poured into the separating funnel and allowed to stand overnight to obtain a clear oil-water interphase.

2.4. Determination of hydrocarbons in oil and WSF

Determination of hydrocarbons in crude oil and WSF was carried out using capillary Gas Chromatography (CGC) at Desert Research Center (Central laboratory), El-Materia, Cairo, Egypt.

2.5. Culture media

2.5.1. Mineral salt medium (MSM) [23]

This medium was used in the isolation of hydrocarbons-degrading microorganisms. Crude oil, water-soluble fraction (WSF) (1%), and anthracene (0.025g/l) soluble in ethanol) were added separately as a carbon source. The pH was adjusted to 6.8.

2.5.2. Modified Dox's agar medium [24]

This medium was used for maintenance of hydrocarbons-degrading fungi. It was modified by replacing sucrose with other carbon sources (crude oil, WSF, and anthracene). The pH was adjusted to 6.8.

2.5.3. Malt extract agar medium (MEA) [25]

This medium was used for maintenance of fungi.

2.5.4. Starch-nitrate agar medium [26]

This medium was used for maintenance actinomycetes.

2.5.5. Nutrient agar medium [27]

This medium was used for maintenance of bacteria.

2.6. Isolation and purification of hydrocarbon-degrading microorganisms

Two methods were used for isolation of hydrocarbons-degrading microorganisms: the direct inoculation method [28] and the serial dilution method [29].

2.7. Purification of microorganisms

2.7.1. Purification of bacteria

A single colony that developed on the surface of the isolation medium was transferred to a Petri dish and repeatedly subcultured until a pure colony was obtained. The purified colonies were then maintained in the refrigerator on nutrient agar slants.

2.7.2. Purification of Actinomycetes

A single colony that developed on the surface of the isolation medium was transferred to a Petri dish and repeatedly subcultured until a pure colony was obtained. The purified colonies were then maintained in the refrigerator on starch nitrate agar slants.

2.7.3. Purification of fungi

Spores or hyphal tips of the isolates were detached and allowed to develop on the agar surface of the sterilized medium. Purity tests were carried out by streaking the isolates on modified Dox's agar medium at the same culture conditions and examined microscopically to ensure the absence of any contamination. The same pure colony was kept on agar slants of the same medium in the refrigerator.

2.8. Identification of hydrocarbons-degrading microorganisms

2.8.1. Gram strain of bacteria according to [30]

2.8.2. The inclined coverslip method according to [31]

2.8.3. Image analysis of fungal isolates

Image analysis was made at Regional Center for Mycology and Biotechnology in Cairo, Egypt, using an Olympus microscope X40 and Olympus microscope at the college.

2.8.4. Genetic identification of fungal isolates

Genetic identification of hydrocarbon-degrading fungi was carried out by analyzing 18S rRNA complete sequencing according to [32] at Macrogen Company for Humanic Genomics in Korea by using primers: Forward (ITS1); 5' (TCC GTA GGT GAA CCT GCG G) 3' and Reverse (ITS4); 5' (TCC TCC GCT TAT TGA TAT GC) 3'.

Phylogenetic analysis of fungal isolates

Evolutionary history was inferred using the UPGMA (unweighted pair group method with arithmetic mean) method [33]. The optimal tree with the sum of branch length = 0.886 is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [34] and are in the units of the number of base substitutions per site. The analytical procedure encompassed 5 coding nucleotide sequences using 1st, 2nd, 3rd, and noncoding positions. The pairwise deletion option was applied to all ambiguous positions for each sequence pair, resulting in a final dataset comprising 1,486 positions. Evolutionary analyses were conducted in MEGA12 [35].

2.9. Screening and selection of the most potent isolates for the biodegradation process.

This stage was made using liquid MSM medium to determine the most potent isolates for the biodegradation process. A set of conical flasks (250 ml capacity) containing 50 ml MSM prepared and sterilized by autoclave at 1.5 atm for 20.0 minutes with 0.5 ml crude oil, 0.5 ml WSF, or 0.5 ml anthracene (soluble in ethanol) as a carbon source. Each flask was inoculated with 2.0 ml of microorganism (three replicates for each microorganism with each carbon source) and incubated at 28±2°C for fungi, 35±2°C for bacteria, and at 33±2°C for actinomycetes.

2.10. Determination of mycelial dry weight

Mycelial dry weight was determined by filtration of growth of microorganisms through dry pre-weighted filter papers, which were washed carefully with distilled water three times for water removal and then dried at 60-70°C to constant weight.

2.11. Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) with a posttest if $P < 0.05$ and using software GraphPad InStat 3.06Guid.

3. Results and Discussion

1. Analysis of soil samples Table 1 represents the physical and chemical analysis of the soil samples used in the isolation of hydrocarbons-degrading microorganisms. The texture of soil sample No. (1) was sandy clay with alkaline pH (8.2), and the texture of sample No. (2) was sand with acidic pH (6.5). The electrical conductivity of sample No. (1) is 3.7 (Ds/m), while that of sample (2) was 10.5 (Ds/m). Sample No. 1 contains 2343.1 mg/l total dissolved solids, whereas sample No. 2 contains 6226.8 mg/l. Many anions and cations, and also many heavy metals, were detected in the two samples. A nil amount of carbonate was recorded in the two soil samples.

Table 1. Physical and chemical properties of soil samples used in the isolation of hydrocarbons-degrading microorganisms.

Soil Sample No.	Physical properties					Chemical properties																									
									Soluble cations mg/l				Soluble anions meq/l				Heavy metals mg/l														
	Coarse Sand %	Fine Sand %	Total Sand %	Silt %	Clay %	pH	E.C. ds/m	TDS mg/l	Ca^{++}	Mg^{++}	Na^{++}	K^{+}	$Co3^{-3}$	HCO_3^{-3}	Cl^{-}	$SO4^{-2}$	Al^{+++}	B^{+++}	Ba^{++}	Si^{++++}	Va^{++}	Zn^{++}	Pb^{++}	Ca^{++}	Co^{++}	Cr^{++}	Cu^{++}	Fe^{++}	Mn^{++}	Mo^{+}	Ni^{++}
1	54.39	6.08	60.47	0.20	39.33	8.2	3.7	2343.1	250.5	100.2	360	21	Nil	134.2	428.5	1115.7	0.6644	2.184	0.0966	10.142	0.581	68.28	8.936	0.097	0.5294	0.4842	0.3516	2.82	0.7504	<0.001	0.9198
2	66.21	27.96	94.17	1.35	4.48	6.5	10.5	6226.8	820.5	202.5	1100	42	Nil	100.7	2999.7	1011.7	4.14	1.06	0.18	12.07	0.5368	1.4034	0.4798	0.0662	0.867	0.391	2.866	9.53	43.58	0.6984	2.802

TDS=Total soluble salts E.C.=Electrical conductivity Soil No.1= DISUCO Soil No.2 Shuker area

2. Determination of crude oil percentages in soil samples.

Table 2 contains the percentages of oil in soil samples used; soil No. (1) contains from 13.4% to 19.4% of oil, while soil No. (2) contains 24.4 to 31.8%. Cooled toluene was superior in the extraction of oil from the two soil samples. The Shuker area, Gulf of Suez Soil, suggests a higher degree of oil contamination. Heavy oils and hydrocarbons may cause persistent contamination, ultimately threaten biodiversity and disrupt ecological balance over the long term.

Table 2. Percentages of oil in soil samples used in this study.

Soil sample	Hexane		Toluene	
	Mean ±SD		Mean ±SD	
	Hot	Cold	Hot	Cold
Soil (1)	0.67 ±0.17 (13.4%)	0.37±0.04 (7.4%)	0.7± 0.09 (14%)	0.97±0.64 (19.4%)
Soil (2)	1.22±0.14 (24.4%)	0.98±0.20 (19.6%)	1.23± 0.05 (24.6%)	1.59±0.21 (31.8%)

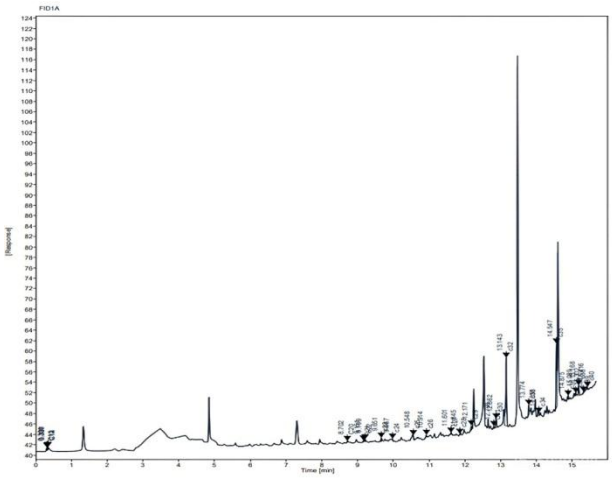

Fig. 1. Hydrocarbons found in the crude oil sample used in this study.

3. Determination of hydrocarbons found in crude oil and WSF used in this study.

The sample of crude oil used in this study as the sole carbon source contains many hydrocarbons (C12, C13, C14 and from C20 to C40), which may be aliphatic (alkane, alkene, and alkyne), aromatic, and polycyclic aromatic hydrocarbons (Table 3). Table 3 contains some suggested names for these compounds. The percentage of hydrocarbons in crude oil or petroleum products varies widely depending on the type of oil, its source, and its composition. Crude oils typically contain a mixture of hydrocarbons, which can range from 20% to over 90% by weight [36]. Common hydrocarbon classes include alkanes, cycloalkanes, aromatics, and alkenes.

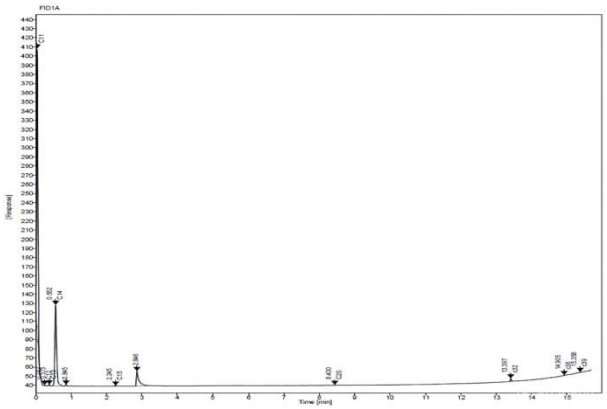

Fig. 2. Hydrocarbons found in the WSF sample used in this study.

Table 3. Hydrocarbons found in crude oil used as sole carbon source in this study.

No.	Hydrocarbons	Retention time	Name
1	C_{12}	0.33	Dodecane or Acenaphthene
2	C_{13}	0.301	Tridecane or Fluorene
3	C_{14}	0.337	Tetradecane or Phenthrene
4	C_{20}	8.702	Eicosane or benzo(a) pyrene
5	C_{21}	9.151	Heneicosane
6	C_{22}	9.199	Docosane or benzo(ghi) perylene
7	C_{23}	9.651	Tricosane
8	C_{24}	9.967	Tetracosane or Coronene
9	C_{25}	10.548	Pentacosane or Pentacontabenzyne
10	C_{26}	10.914	Hexacosane or Hexanelicene
11	C_{27}	11.601	Heptacosane
12	C_{28}	11.845	Octacosane
13	C_{29}	12.171	Nonacosane
14	C_{30}	12.862	Triacosane or Triaconta hexaene
15	C_{31}	12.798	Hentriacontane
16	C_{32}	13.143	Dotriacontane or Ovalene
17	C_{33}	13.774	Tritriacontane
18	C_{34}	14.056	Tetratriacontane or hexabenzo[bc,ef,hi,kl,no,qr] coronene
19	C_{35}	14.547	Pentatriacontane
20	C_{36}	14.875	Hexatriacontane
21	C_{37}	15.089	Heptatriacontane
22	C_{38}	15.168	Octatriacontane
23	C_{39}	15.302	Nonatriacontane
24	C_{40}	15.416	Tetracontane or Tetranaphthylene

The sample of water-soluble fraction (WSF) contains hydrocarbons that are soluble in water, which are represented by C12, C13, C14, C15, C19, C32, C35 and C39, in Table 4. High percentages of C19, C32, and C35, names of these hydrocarbons, may be as recorded in Table 4. [37] concluded that the WSF of petroleum and its derivative products contains a mixture of polycyclic aromatic hydrocarbons (PAH); monoaromatic hydrocarbons, often referred to as BTEX (benzene, toluene, ethylbenzene, and xylenes); phenols; and heterocyclic compounds containing nitrogen and sulfur.

4. Isolation of hydrocarbon-degrading microorganisms

A total of nine bacterial isolates were obtained from the two soil samples used in this study (Table 5). The bacterial isolates developed within a period of 4 to 6 days of incubation at a temperature of 35±2°C, during which visible bacterial colonies were observed on the Petri dishes. Four isolates were derived from soil sample No. (2) on MSM. Conversely, five isolates were obtained from soil sample No. (1) on MSM medium. Thir-

ty-two isolates were isolated by [38] oil broth and agar media.

Twelve actinomycete isolates were recovered from the two soil samples (Table 6). Nine isolates were isolated from soil sample No. (2), and three samples were isolated from soil sample No. (1) on MSM medium. **Olajuyigbe and Ehiosun** [39] isolated eighteen isolates from different soil depths (20–120 cm) were screened for their ability to grow on crude oil–based medium (COBM). Regarding fungi, ten isolates were obtained from the two soil samples (Table 7). A total of five isolates were collected from each soil sample on MSM. [40] isolated twenty-three fungal isolates using Czapek's broth medium containing crude oil.

Table 4. Hydrocarbons found in water soluble function sample used as a carbon source in this study.

No.	Hydrocarbons	Retention time	Name
1	C_{12}	0.243	Dodecane or Acenaphthene
2	C_{13}	0.373	Tridecane or Fluorene
3	C_{14}	0.552	Tetradecane or Phenanthrene
4	C_{15}	2.245	Pentadecane
5	C_{19}	6.337	Nonadecane
6	C_{32}	13.397	Dotriacontane or Ovalene
7	C_{35}	14.318	Pentatriacontane
8	C_{39}	15.358	Nonatriacontane

Table 5. Bacterial isolates, isolated from different soil samples on MSM medium, and some primary characters* on nutrient agar medium.

Isolate number	Colony color	Medium of isolation	Medium of characterization	Soil sample	Gram Reaction and form of bacteria
1	Creamy	MSM	Nutrient agar	2	+Ve, bacilli, spore forming
2	Creamy	MSM	Nutrient agar	2	+Ve, bacilli, spore forming (sub terminal)
3	White	MSM	Nutrient agar	1	+Ve, Streptobacilli
4	Yellow	MSM	Nutrient agar	1	+Ve, spore forming bacilli.
5	Creamy	MSM	Nutrient agar	2	+Ve, mono bacillus
6	Creamy	MSM	Nutrient agar	2	+Ve, Streptobacilli, spore forming
7	Creamy	MSM	Nutrient agar	1	+Ve, Streptobacilli, spore forming
8	Creamy	MSM	Nutrient agar	1	+Ve, diplobacilli
9	Creamy	MSM	Nutrient agar	1	+Ve, streptobacilli

*(Colony color, soil sample and gram reaction and bacterial form).

Table 6. Actinomycete isolates, isolated from different soil samples on MSM medium, and some primary characters* on starch nitrate agar medium.

Isolate number	Colony color	Colony surface	Medium of isolation	Medium Of characterization	Soil Sample
1	Dark gray	Smooth	MSM	Starch nitrate	2
2	Off white	Smooth	MSM	Starch nitrate	2
3	Golden	Rough	MSM	Starch nitrate	2
4	White	Granular	MSM	Starch nitrate	1
5	Light gray	Smooth	MSM	Starch nitrate	2
6	Dark gray	Granular	MSM	Starch nitrate	2
7	Golden	Granular	MSM	Starch nitrate	2
8	Gray	Rough	MSM	Starch nitrate	1
9	White	Smooth	MSM	Starch nitrate	2
10	Off white	Rough	MSM	Starch nitrate	2
11	Gray	Smooth	MSM	Starch nitrate	1
12	White	Smooth	MSM	Starch nitrate	2

*(colony color, colony surface, media of isolation and soil sample)

Table 7. Fungal isolates, isolated from different soil samples on MSM medium, and some primary characters* on Malt extract agar medium.

Isolate number	Colony Color	Colony surface	Medium of isolation	Medium of characterization	Soil sample
1	Light golden	Rough	MSM	Malt extract agar	1
2	Gray	Rough	MSM	Malt extract agar	1
3	Brown	Rough	MSM	Malt extract agar	2
4	Dark brown	Smooth	MSM	Malt extract agar	2
5	White	Cottony	MSM	Malt extract agar	2
6	Yellow	Smooth	MSM	Malt extract agar	1
7	Dark green	Smooth	MSM	Malt extract agar	2
8	Dark brown	Rough	MSM	Malt extract agar	1
9	Dark green	Smooth	MSM	Malt extract agar	1
10	Dark brown	Rough	MSM	Malt extract agar	2

*(colony color and colony surface).

5. Screening and selection of the most potent microbes for the hydrocarbon biodegradation process.

All the isolated microorganisms successfully grew on the hydrocarbons provided, which suggests their potential capability to utilize hydrocarbons as a sole carbon source. Using liquid medium to identify the most potent microorganism in each group is a logical approach, as it allows for a controlled environment to compare growth efficiency, which indicates the hydrocarbon degradation ability. Figures (3, 4 and 5) present comparable data sets, each illustrating the microbial biomass (mg/100 ml culture medium) across different microbial groups, bacteria, actinomycetes, and fungi (respectively) grown on three hydrocarbons: oil, WSF, and anthracene.

In bacteria (Fig. 3) generally high biomass was obtained by isolates 8 and 9 on oil, while in actinomycetes (Fig. 4), the high biomass was obtained by isolates 3, 11, and 12 on oil. Whereas fungi (Fig. 5) show consistent high growth, especially by isolates 2, 4, and 6 on oil also.

The microbial growth of the three groups on WSF was, for bacterial isolates (isolates 4 and 7), good growth, and actinomycetes have moderate growth; the most potent ones are isolates 4 and 8.

In the case of fungi, it is moderate but relatively high; isolates No. 2 and 10 show strong growth. WSF supports growth but generally less than oil. Anthracene is the least preferred substrate across all microbial groups: bacteria, actinomycetes, and fungi. Oil tends to be more suitable for growth compared to other hydrocarbons (WSF and Anthracene). This may be due to high energy density of oil, meaning it provides a large amount of energy per unit volume or mass.

Among microorganisms, bacteria have been identified as the main degraders and the most active substances in the breakdown of petroleum contaminants [41]. The nocardioform actinomycetes (e.g., *Rhodococcus*, *Gordonia*, and *Mycobacterium*), which are known as hydrocarbons degraders that have been isolated by many workers [42-44] and degrade PAH (polycyclic aromatic hydrocarbons) in soil. Among fungi [45, 46, 47, 48], it was recorded that *Aspergillus* and *Penicillium* species were the most efficient genera in degrading hydrocarbons.

Fungal isolates generally show high biomass, suggesting fungi might be particularly effective for hydrocarbon degradation. So fungal isolates No. 2, 4, 5, 6 and 10 were used to continue this search.

6. Identification of most potent fungal isolates.

6.1. Morphological identification of most potent fungal isolates

An image analysis of the most potent fungal isolates:

Fungal isolate No. 2: Colonies on CYA attending 3-5 cm diameter at 25°C, radially, white, grayish, buff to green. Yellow soluble pigment produced and yellowish-brown reverse. No growth on CYA at 5°C and 37°C. The microscopic examination revealed the characterization of Penicillium type, Bi-verticillate, and sometimes terverticillate, conidiophore diameter 2.8 µm; Rami 21.0X3.7 µm; metulae 12X3.0 µm; phialides 9.0 X 2.2 µm and conidia, ellipsoidal 2.5X2.0 µm. This fungus resembles *Penicillium sp*.

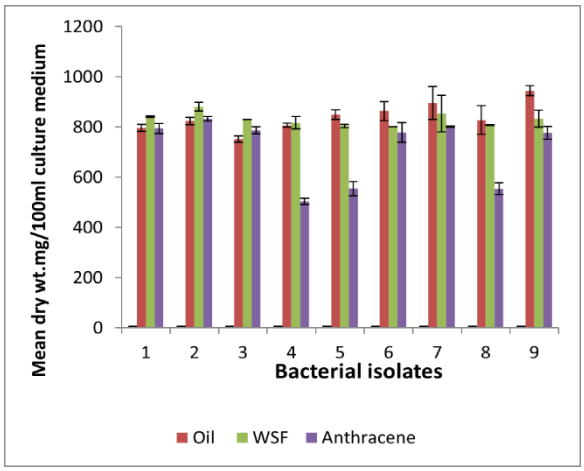

Fig. 3. Dry weight of bacterial isolates cultivated on MSM medium amended with different hydrocarbons.

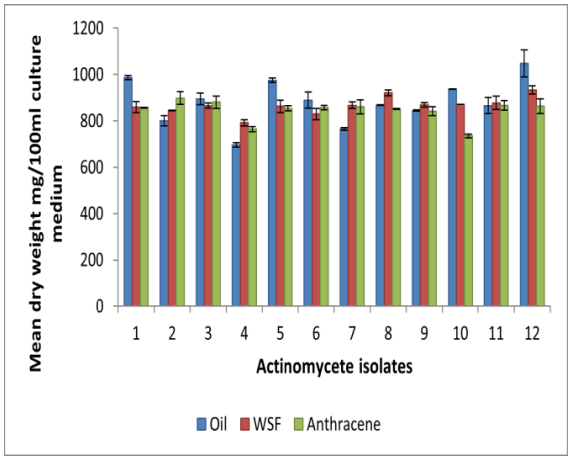

Fig. 4. Dry weight of actinomycetes cultivated on MSM medium amended with different hydrocarbons used

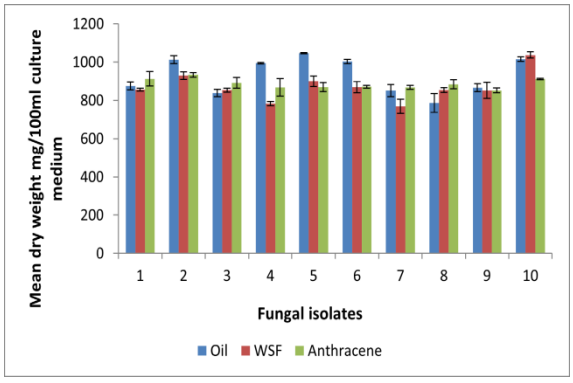

Fig. 5. Dry weight of fungal isolates cultivated on MSM medium amended with different hydrocarbons.

Fig. 6. Images of fungal isolate No.2

Fungal isolate No. 4: Image analysis of fungal isolate No. 4 revealed that it has colonies of diameter 5.5 cm, conidiophores 6 µm in diameter, and ellipsoidal, clavate, brown conidia with rounded ends 19 X 8.5 µm. This fungus resembles *Curvularia* sp.

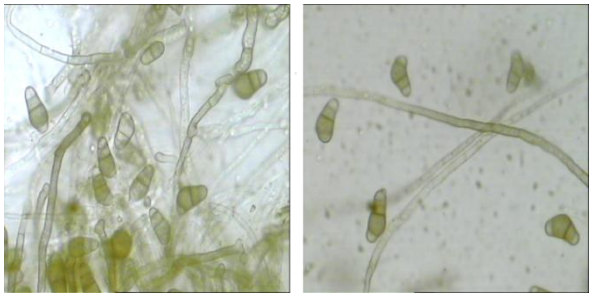

Fig. 7. Images of fungal isolate No.4

Fungal isolate No. 5: Image analysis of fungal isolate No. 5 revealed that it has colonies on PDA attaining a diameter of 3.0 cm in 4 days, with whitish mycelium with bluish pigment. Micro-conidia are 0-1 septate and variable in shape (ovoid), 7.0X2.8 µm; macroconidia are 1-3 septate, fusiform, and pointed at both ends with a pedicellate basal cell 25.0X4.0 µm; and Chlamydospores are hyaline, subglobose, terminal or intercalary, and 12.0 µm. This fungus resembles *Fusarium* sp.

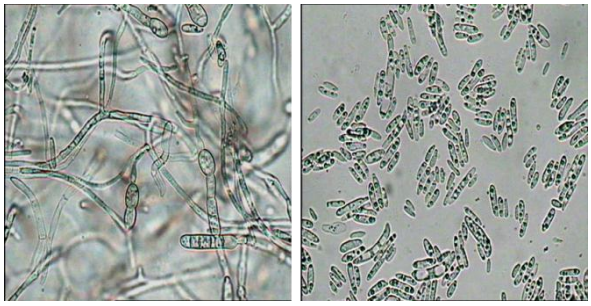

Fig. 8. Images of fungal isolate No.5.

Fungal isolate No. 6: Image analysis of fungal isolate No. 6 revealed that it has colonies of diameter 5-7 cm, black, radiate conidial heads, conidiophores 8.0 µm in diameter, and globose-subglobose, 25.0 µm vesicles. Sterigmata may be either a single or double series (9.5 X 5.0 µm, 8.0 X 3.0 µm second sterigmata) and conidia globose (4.5 µm). This fungus resembles *Aspergillus sp.*

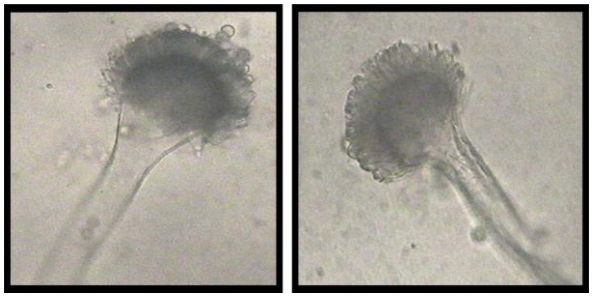

Fig. 9. Images of fungal isolate No.6.

Fungal isolate No. 10: The image analysis of fungal isolate No. 10 revealed that it has colonies reaching 5-7 cm in diameter in 7 days at 28°C on Czapek's, velvety, black colonies; reverse colorless to pale yellow; black, radiate conidial heads; conidiophore 12.5 µm in diameter; a vesicle that is globose-subglobose, 23.0 µm; conidia globose, 3.5 µm; a first stigmata of 13.7 X 5.5 µm; and a second stigmata of 7.0 X 3.0 µm. This fungus resembles *Aspergillus* sp.

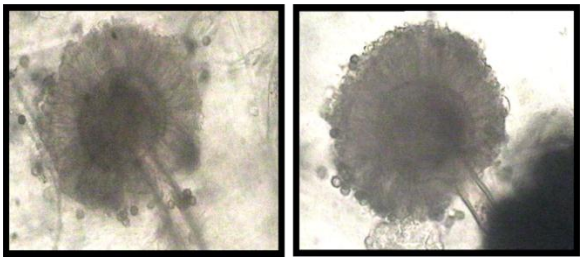

Fig. 10. Images of fungal isolate No.10.

6.2. Genetic identification of the hydrocarbons-degrading fungal isolates

Table (8) showed the BLAST results of the most potent five fungal isolates, which reveals that the gray isolate No. (2) was *Talaromyces wortmannii,* which is related to *Penicillium roqueforti* by 96.59%, and the brown isolate No. (4) was *Curvularia geniculate,* and it was related to *Curvularia lunata* by 97.07% identity. The isolate No. 5 was white-colored *Fusarium oxysporum* by identity 100%*;* it was related to other *Fusarium oxysporum* by 99.43. The two other fungal isolates were *Aspergillus* sp. the yellow isolate No. (6) was *Aspergillus oryzae* by 100% identity, and it was related to *Aspergillus flavus* and *Aspergillus niger* by 96.21 and 96.46 identity, respectively. The black isolate No. (10) was related to *Aspergillus niger* by 100% identity.

Table 8. Blast results of the 18S ribosomal RNA gene, partial sequence of identified fungal isolates.

No. of isolate	Scientific Name	Max Score	Total Score	Query Cover	Per. Ident%	Acc. Len	Accession
2	*Talaromyces wortmannii*	2529	2529	100%	100.0	1369	PQ394979.1
	Penicillium roqueforti	2274	2274	100%	96.59	1775	MT544459.1
	Penicillium sp.	2274	2274	100%	96.59	1662	KX906964.1
4	*Curvularia geniculata*	2512	2512	100%	100.00	1360	PQ394985.1
	Curvularia lunata	2283	2283	99%	97.07	1754	JN941608.1
	Bipolaris sp.	2283	2283	99%	97.07	1731	KX852423.1
5	*Fusarium oxysporum*	2298	2298	100%	100.00	1244	PQ394987.1
	Fusarium oxysporum	2222	2222	98%	99.43	1613	MF376147.1
	Fusarium oxysporum	2220	2220	97%	99.75	1655	MZ501950.1
6	*Aspergillus oryzae*	2523	2523	100%	100.00	1366	PQ394988.1
	Aspergillus flavus	2137	2137	95%	96.21	1344	KY233188.1
	Aspergillus niger	2135	2135	95%	96.46	1300	MN559756.1
10	*Aspergillus niger*	2134	2134	100%	100.0	1155	PQ394996.1
	Aspergillus welwitschiae	2134	2134	100%	100.0	1699	OL711714.1
	Aspergillus niger	2134	2134	100%	100.0	1736	KJ365316.1

The morphological identification was consistent with the results of the genetic identification. The GenBank accession numbers of the fungal isolates obtained in this study are listed in Table (9).

The constructed phylogenetic tree (Fig. 11) indicated the phylogenetic position of the isolates. The bootstrap (evolutionary distance) values supported that different strains of isolates are closely related with known strains from Gene bank. However, the bootstrap values were linked with morphological features to confirm the close relationship between the isolates.

Table 9. GenBank accession numbers of fungal isolates isolated in this study.

No. of isolate	Accession No	Name of fungal isolate
2	PQ394979	*Talaromyces wortmannii*
4	PQ394985	*Curvularia geniculata*
5	PQ394987	*Fusarium oxysporum*
6	PQ394988	*Aspergillus oryzae*
10	PQ394996	*Aspergillus niger*

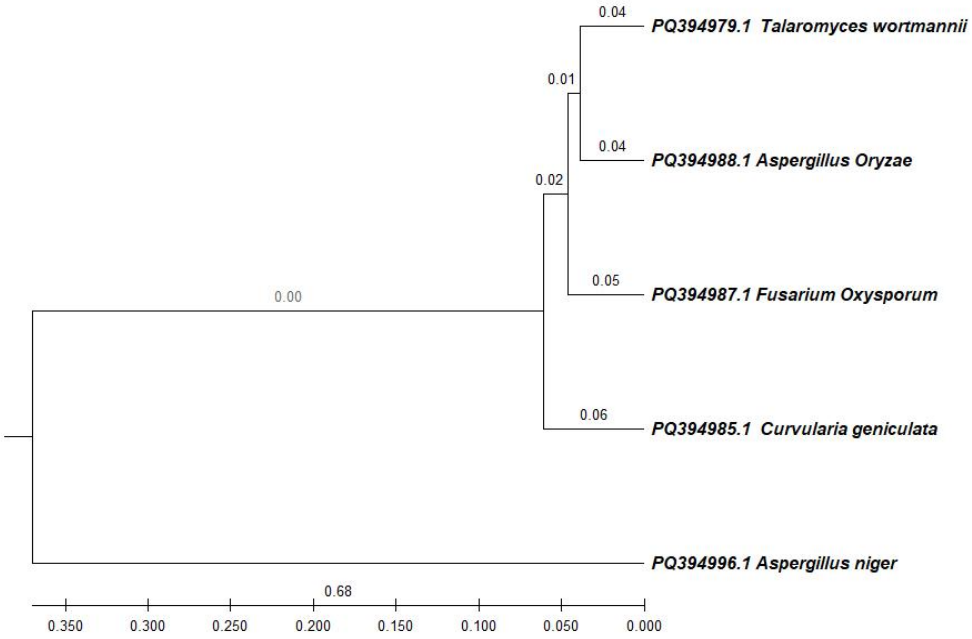

Fig. 11. Rooted phylogenetic tree created using the neighbor-joining method and is based on a comparison of the 18S ribosomal RNA sequences of five fungal isolates and their closest phylogenetic relatives.

7. Conclusion

Fungi are key agents in environmental biodegradation, contributing significantly to the renewal of ecosystems and the regulation of the global carbon cycle. They have the remarkable ability to degrade or transform various pollutants, including petroleum hydrocarbons and other chemicals that pose ecotoxicological risks. The Gulf of Suez and Kafr El-Sheikh soils harbor several promising microbial species that could be

employed in the biodegradation of petroleum hydrocarbons, offering a safe alternative for environmental remediation.

Recommendation

Various microbial species present in the soil, particularly fungi, demonstrate the ability to metabolize petroleum and aromatic hydrocarbons, making them valuable agents for eco-friendly bioremediation efforts. *Talaromyces wortmannii, Curvularia geniculata, Fusarium oxysporum, Aspergillus oryzae, and Aspergillus niger* are recommended. These fungal isolates can be employed to clean or eliminate petroleum contaminants from soil in the DISUCO company area in Kafr El-Sheikh Governorate and in the Shuker area in Suez.

The next step involves optimizing various parameters that influence the biodegradation process, as well as analyzing the hydrocarbons present in the filtrate of the most potent fungal isolates and in the filtrate of mixed cultures of the most potent fungal isolates with different carbon sources, using Capillary Gas Chromatography (CGC).

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