ISOLATION AND CHARACTERIZATION OF OIL UTILIZING BACTERIA ISOLATED FROM DRILLING OILY BASE MUD WASTE IN THE EGYPTIAN WESTERN DESERT

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ABSTRACT

The main aim of this study is to isolate, screen and identify the most potent oil degrading bacteria (ODB) isolated from oil base mud (OBM) waste resulted from group of selected oil and gas drilling and exploration wells. Another aim is to determine the metabolic fingerprint of the most potent ODB. To achieve these aims, eleven samples from the waste of oily base mud were collected from different drilling sites at western desert. Total bacterial counts at 22 oC and 30 oC were determined for giving a full picture about the presence of natural bacterial flora in the collected samples. Moreover, ODB were determined on oil agar media containing 0.1% oil (V/V) using spread plate method. The results showed that, the average of total bacterial counts at 22 oC and 30 oC were 1.4x104 and 1.6x104 CFU/g, respectively. Moreover, the oil degrading bacteria (ODB) counts were fluctuated between 1.0×102 and 6.1x103 CFU/g in all collected samples. After six screening steps, the most potent oil degrading bacteria were identified using BIOLOG as follows Enterobacter hormaechei, Enterobacter cloacae and Bacillus subtilis. The only difference in the metabolic fingerprints view between two bacterial strains that, Enterobacter hormaechei (H17) are able to utilize D-serine while and Enterobacter cloacae (H32) not. It can be concluded that, metabolic fingerprints of the most potent oil degrading bacteria provide full information

about the microbial metabolic activities that may be helpful in determination of favorable conditions which aid in biodegradation experiments.
Keywords and phrases: Oil base mud (OBM), drilling waste, Oil Degrading Bacteria (ODB), western desert, Egypt.

INTRODUCTION

The Egyptian oil and gas industries provide power and main chemicals for houses, manufactures, and transportations, and so have a very important role in the Egyptian economy. It supplies around 87% of Egypt's primary energy and accounts for about 35% of Egypt's export revenues (EIA, 2013).

Egypt is the biggest oil producer in Africa not a member in the Organization of the Petroleum Exporting Countries (OPEC) and the second-biggest natural gas producer on the continent, but Egypt also has the third largest population in Africa according to the World Bank (WBD, 2015 and EIA, 2015). Because of the population; Egypt also is the largest oil and natural gas consumer in Africa, with about 20% of petroleum and other liquids consumption and 40% of dry natural gas consumption in Africa in 2013. Egypt's total main energy consumption was 1.7 million barrels per day (b/d), according to the (BP, 2014).

The Western Desert of Egypt covers two thirds of the whole area of Egypt, and is considered the most productive petroleum region in Egypt. Many studies refer that approximately 90 % of oil and 80 % of gas reserves have not yet discovered in the Western Desert (EGPC 1992). The coastal basins (Matruh, Shushan, Alamein and Natrun) located in the Northern area of the Western Desert 75 kilometers to the southwest of Matruh City cover an area of about 3800 Km2 which makes the main part of the unstable shelf. It is

located northeast-southwest trending basin. This basin is characterized by its great oil and gas accumulations and its oil productivity about 45,000 BOPD from 150 producing wells in 16 oilfields. This represents more than 33% of the oil production from the Northern Western Desert of Egypt (EGPC, 1992).

Over time, the oil well drilling industry has basically made use of two types of drilling fluids, namely, water-based muds (WBMs) and oil-based drilling muds (OBMs). On the one hand, due to the lower costs and ease of formulation, WBMs are most commonly used as drilling fluids. On the other hand, the OBMs regardless of being more costly compared with their water based rivals; OBMs have the advantage of being able to drill through formations containing water swell-able clays. To formulate these OBMs, diesel oil is used as the base fluid primarily because of its viscosity characteristics, low flammability, and low solvency for rubber (Bourgoyne et al., 2003). All this petroleum-based oils used for drilling mud contain relatively large amounts of aromatics and at least a substantial concentration of n-olefins both of which may be harmful or toxic to animal and plant life (Dardira *et al*., 2014). However, most great impacts arise from the discharge of wastes into the environment in concentration that is not naturally found in such environment. The wastes created during hydrocarbon production can be largely divided into liquids (i.e produced water & oil) and solids (i.e drilling muds & cuttings) (Ferrari *et al*., 2000).

In general, drilling wastes include formation cuttings and drilling fluids (drilling Muds) with different kinds of chemical additives depending on the type of the drilling fluid that have been used. When oil-based or synthetic-base drilling fluids are used, the hydrocarbon content in the discharged

cuttings will be of 10 to 15 % beside some of other residual weighting materials. These cuttings need specialized treatment before the appropriate disposal (Al-Joubori and Abd Al-Razaq, 2008).

Operators have employed a variety of methods for managing these drilling wastes depending on what federal regulations allow and how costly those options are for the well in question. These include land application (Onsite Burial and Land farming), underground injection, Incineration, thermal treatment, and biological remediation (Onwukwe and Nwakaudu, 2012).

Biological treatment processes are currently in popularity as appropriate, cost-effective and performance-effective technology to classify numerous environmental pollution problems. One of the efficient and environmental friendly procedures used in oily waste treatment is the use of biological agents such as petroleum hydrocarbon degrading microbes which are known as bioremediation process (Helmy and Kardena, 2015)

Therefore, this study aims to isolate, screen and identify oil degrading bacteria isolated from oil base mud. Moreover, to assess the phenotypic characteristics and metabolic fingerprint for this oil degrading bacteria to give a complete picture on the survival, distribution and behavior of these microorganisms that will be used in further studies for biodegradation.

MATERIAL AND METHODS

Eleven oil base mud wastes were collected from different sites in Abu Sennan, El Alamein, Qattara Depression, Burg El Arab, Marina, and El Hamra. All these sites are located at western desert, Egypt. The samples were

preserved in ice box and transferred to the laboratory according to (APHA, 2017) for bacteriological examination.

1. **Enumeration of total bacterial counts:**Enumeration of total bacterial counts was carried out at two different incubation temperatures (22oC and 30°C). Total bacterial counts were determined by using pour plate method according to (APHA, 2017). Totally 10 g of each sample was suspended in 90 ml sterile distilled water and vortex for 5 min. Then ten fold serial dilutions from each suspension were done. One ml from suitable dilution was plated on plate count agar medium (Difco™) in a sterile Petri dish. The plate count agar medium supplemented with 50 µg/ml nystatin to inhibit fungal growth (Williams and Gray, 1973). Two plates were incubated at 30±0.5°C for 24 h and one un-inoculated plate served as a control. Also, two plates were incubated at 22±0.5°C for 48 h and un-inoculated plate served as a control. After the incubation period, all plates were counted with the use of digital colony counter.
2. **Enumeration of Oil Degrading Bacteria (ODB):** ODB were enumerated using spread method on oil agar (OA) medium (Ijah and Antai, 2003). The oil was purchased from Cooperative Company for petrol (CoOP) in Egypt. Oil agar (OA) medium was prepared and its pH was adjusted before sterilization. After oil sterilization, one mL from oil was added as a sole carbon source into one liter of sterilized oil agar medium. The media was poured in sterile Petri dishes; 0.1 mL of the serially diluted oil base mud sample was spread on the surface of media. The plates were incubated at 30°C for 5 days before taking the counts (Ijah and Antai, 2003).

3. **Isolation and Purification of Oil Degrading Bacteria (ODB):** Oil degrading bacterial colonies on oil agar (OA) surface medium were picked and purified by re-streaking twice times on the same culture agar medium (OA) before storage at -20°C on tryptic soy broth (TSB) (Difco™) for further screening.
4. **Screening of oil degrading bacterial (ODB) isolates:** The ability of the bacterial isolates to utilize oil as source of carbon and energy was determined according to (Okpokwasili and Okorie, 1988). 0.1 mL from the TSB culture (Difco™) of fresh degrading isolates was inoculated into test tube containing 10 mL of sterile mineral salts medium (MSM) of Bushnell and Haas (Luning and Pritchard, 2002; Xia et al., 2006), then added 0.1% (V/V) oil. Control test tube was prepared by using 10 mL of MSM with 0.1% (V/V) oil, but had no added bacteria. The screening was repeated with the same method at different oil concentrations 0.2, 0.3, 0.4, 0.5 and 0.6% (V/V). The tubes were incubated at room temperature for five days according to (Das and Mukherjee, 2007). The oil degrading bacterial growth was assessed by streaking on surface of oil agar (OA) media and plate count agar from the previously inoculated MSM media with tested isolates. The oil agar and plate count plates were incubated at 30°C for 5 days and 24 h, respectively.
5. **Phenotypic identification of the most three potent oil degrading bacterial (ODB) isolates using BIOLOG GEN III:** After the six screening steps, the most three potent oil degrading bacteria that are able

to degrade the highest oil concentrations were identified using BIOLOG GEN III as phenotypic.

BIOLOG GEN III is considered as suitable tool for phenotypic tests that contain 71 carbon source and 23 chemical sensitivity assays on a microplate in one shot. The test panel provides a phenotypic fingerprint of the tested microbes.

Preparations of the three stocked oil degrading bacterial isolates for phenotyping identification were carried out according to (El-Liethy *et al.* 2018). Isolates which previously kept in 10 % glycerol in -20oC were inoculated into Tryptic Soy Broth (TSB) and incubated at 37 °C for 24 h. A loopful of the 24 h culture was streaked into Tryptic Soy Agar (TSA) (BD, Germany) plates and incubated at 37 °C for 24 h. Following incubation, a single colony was picked using a sterile disposable inoculator swab and inoculated into 10 ml of inoculating fluid (IF-A) (Biolog Inc., USA). The inoculated IF-A was dispensed into 96 wells of a microplate (100 µl per well) using a multichannel repeating pipettor. The microplate was incubated at 37 °C for 24 h. The tetrazolium violet as a color indicator was responded to the metabolic processes by changing from colorless into purple in the positive wells. The results were read after 6 and 24 hours of incubation. The reading was carried out automatically by the computerized MicroStation™ system (Biolog Inc., USA) with the fingerprint data which was previously fed into the software (OmniLog® Data Collection) and used to identify the bacteria from their phenotypic patterns in the GEN III MicroPlate.

RESULTS AND DISCUSSION

1) **Enumeration of total bacterial counts and oil degrading bacteria from oil base mud samples:** In this study, total bacterial counts were enumerated at two incubation temperatures (22^{o}C and 30^{o}C) in the collected oil base mud samples for providing a complete picture about presence of the natural bacterial flora in the samples. It was observed that, total bacterial counts at 22^{o}C were higher than that at 30^{o}C in oil based mud samples (Table 1 and Figure 1). This means that temperature at 22^{o}C was the most suitable temperature for natural bacterial growth.

The average of total bacterial counts at 22^{o}C and 30^{o}C were 1.4x10^{4} and 1.6x10^{4} CFU/g, respectively. The minimum counts of total bacteria were observed at site 5. Otherwise, the highest counts of the total bacteria were observed at site 8 at both 22^{o}C and 30^{o}C (Table 1 and Figure 1). In another study carried out by (*Imarhiagbe and Atuanya, 2014*), they determined the total bacterial counts by pour plate method in drill mud samples. They found that, the mean of the total bacterial counts ranged between 5.4x10^{5} CFU/g and 7.23x10^{5} CFU/g. The highest recorded drill mud utilizing bacterial counts was 7.7x10^{3} CFU/g for medium amended with water based mud while the least drill mud utilizing bacterial counts (3.1x 10^{2} CFU/g) was recorded for medium amended with non-aqueous drilling mud. The total bacterial count at 37^{o}C in sediment samples collected from highly oil polluted area in Isamlia Canal were fluctuated between 4.9×10^{3} and 3.7×10^{6} CFU/g. Moreover, The TBC at 22ºC was fluctuated between 2.7×10^{4} and 5.4×10^{6} CFU/g (Yossef, 2010).

2) Enumeration of Oil Degrading Bacteria (ODB):

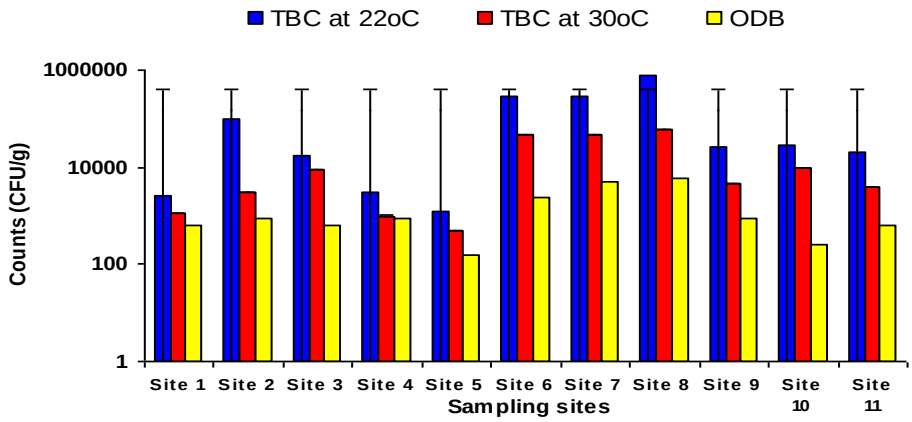
In this study, oil degrading bacteria counts (ODB) were determined by using surface plate method on oil agar (OA) media in oil base mud samples collected from eleven sites located at Eastern desert, Egypt. The ODB counts were fluctuated between 1.0×10^2 and $6.1x10^3$ CFU/g (Table 1 and Figures 1 and 2). The highest ODB counts were observed at sites 6, 7 and 8. In another study by (Balogun *et al*., 2014), they found that the total bacterial counts in auto-mechanic workshops samples ranged from 1.0×10^6 to 2.8×10^6 CFU/g. Moreover, total oil degrading bacterial counts varied between 4.0×10^5 and 2.0×10^6 CFU/g. (Yossef, 2010) found that, the average of the count on OA over the period of one year for sediment samples collected from Ismailia Canal as River Nile Branch was 4.3×10^4 CFU/g.

In some studies, it was found that higher ratios of oil degrading bacteria to total bacterial counts in the active Ekofisk oil field of the North Sea. These may be due to the occurrence of oil pollutants in the sediment of this region (Oppenheimer *et al*., 1977; Atlas, 1981; Yossef, 2010, El-Liethy *et al*., 2017). In that study the percentages of ODB in total bacterial counts at 30^oC ranged between 3.0 to 90%. Sampling site (4) contains the higher percentage (90%) of ODB followed by site (1) contains 59% ODB in total bacterial counts (Table 1).

Statistically, there was positive highly significant correlation between total bacterial counts at 22^oC and ODB counts ($P>0.01$). Moreover, there was positive significant correlation between total bacterial counts at 22^oC and at 30^oC ($P>0.05$). Also, there was positive significant correlation between total bacterial counts at 30^oC and ODB counts ($P>0.05$).

Table(1) : Total bacterial count (CFU/g) at 22 oC and 30°C and oil degrading bacteria (CFU/g) at 30°C in oil based mud samples.

Sample number	Total bacterial count (TBC) (CFU/g) at		Oil degrading bacteria (ODB) (CFU/g) at 30oC	% of ODB in total bacterial count at 30oC
	22oC	30oC		
S-1	2.6x103	1.1x103	6.5x102	59
S-2	9.9x104	3.0x103	9.0x102	30
S-3	1.8x104	9.1x103	6.3x102	7.0
S-4	3.0x103	1.0x103	9.0x102	90
S-5	1.2x103	5.0x102	1.0x102	20
S-6	2.9x105	4.5x104	2.3x103	5.0
S-7	3.0x105	4.8x104	5.2x103	11
S-8	8.0x105	6.0x104	6.1x103	10
S-9	2.6x104	4.8x103	9.0x102	19
S-10	2.8x104	9.6x103	2.5x102	3.0
S-11	2.0x104	4.0x103	6.1x102	15
Min.	1.2x103	5.0x102	1.0x102	20
Max.	8.0x105	6.0x104	6.1x103	10
Average	1.4x104	1.6x104	1.6x103	10



Figure(1): Total bacterial counts (TBC) at 22oC and 30oC and Oil Degrading Bacteria (ODB) (CFU/g) in the collected oil base mud samples

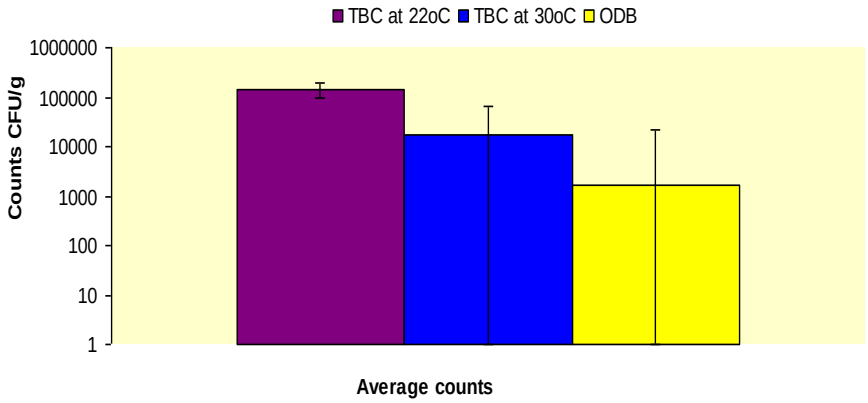


Figure (2): Average counts of total bacteria at 22oC and 30oC and oil degrading bacteria in oil base mud samples

3) **Isolation and screening of oil degrading bacteria (ODB) from oil base mud samples:** In this study, forty five oil degrading bacterial isolates were purified and screened six times on different oil concentrations (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 (V/V) (Tables 2 and 3). After first screening step, 34 out of total 45 (75.5%) oil degrading isolates were able to grow on media containing 0.1(V/V) oil. After second screening step, 30 out of total 45 (66.6%) oil degrading isolates were able to grow on media containing 0.2 (V/V) oil. After third screening step, 19 out of total 45 (42.2%) oil degrading isolates were able to grow on media containing 0.3 (V/V) oil. After forth screening step, 11 out of total 45 (24.4%) oil degrading isolates were able to grow on media containing 0.4 (V/V) oil. After fifth screening step, only 9 out of total 45 (20%) oil degrading isolates were able to grow on media containing 0.5 (V/V) oil. Finally after six screening step, three oil degrading bacterial isolates were selected as the most efficient oil degrading bacteria coded H17, H32 and H43 and able to grow on media

containing 0.6 (V/V) oil (Tables 2 and 3, figure 3). (El-Liethy *et al.,* 2017) found after four frequent screenings, oil degrading bacteria were identified out of 225 isolates. They found the most potent oil degrading bacteria was Pseudomonas aeruginosa, Coronobacter sakazakii, klebsiella oxytoca and Bordetella bronchispetica consequently. The results demonstrated that, the increase in oil concentration leads to decrease the number of oil degrading bacterial isolates in screening steps. This coincides with (Rambeloarisoa *et al*., 1984) who reported that, the degradation is inversely proportional to the concentration of oil. (Zhang *et al.,* 2005) found that, the initial concentration of 0.7 g/L in mineral salt medium in the presence of 1 g/L glycerol and 0.22 g/L rhamnolipids led to 58 and 60% degradation of oil, respectively. Also, (Sun *et al.,* 2009) declared that, a quite fast degradation present with low concentration of oil, while in case of increasing of oil concentrations, the biodegradation rate becomes slower then suppressed.

Table (2): Number and percentage of positive oil degrading bacterial isolated after six screening steps at different oil concentrations

Total ODB isolates	**Oil Concentrations screening steps (V/V)**											
	First (0.1)		**Second (0.2)**		**Third (0.3)**		**Fourth (0.4)**		**Fifth (0.5)**		**Sixth (0.6)**	
	+	**%**	**+**	**%**	**+**	**%**	**+**	**%**	**+**	**%**	**+**	**%**
45	34	75.5	30	66.6	19	42.2	11	24.4	9	20	3	6.6

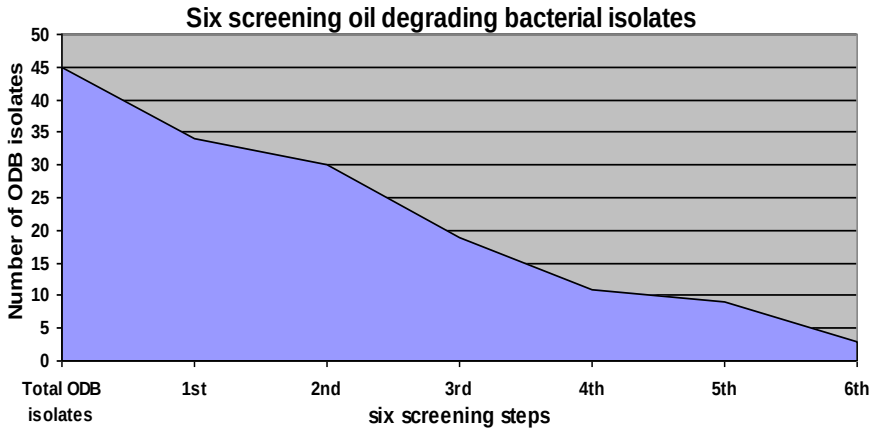


Figure (3): Number of positive oil degrading bacterial isolated after six screening steps at different oil concentrations

Table (3): Six screening steps of oil degrading bacteria on oil agar media

Isolates code	Oil concentrations (V/V)					
	0.1	0.2	0.3	0.4	0.5	0.6
H1	+	+	-	-	-	-
H2	+	+	-	-	-	-
H3	-	-	-	-	-	-
H4	+	-	-	-	-	-
H5	-	-	-	-	-	-
H6	+	+	+	-	-	-
H7	-	-	-	-	-	-
H8	+	+	+	-	-	-
H9	+	+	+	-	-	-
H10	-	-	-	-	-	-
H11	+	-	-	-	-	-
H12	+	+	-	-	-	-
H13	-	-	-	-	-	-
H14	-	-	-	-	-	-
H15	-	-	-	-	-	-
H16	-	-	-	-	-	-
H17	+	+	+	+	+	+
H18	+	+	-	-	-	-
H19	+	+	-	-	-	-
H20	+	+	-	-	-	-
H21	+	+	+	+	+	-
H22	+	+	+	+	+	-
H23	-	-	-	-	-	-
H24	-	-	-	-	-	-
H25	+	+	+	+	+	-

Table (3): Continue. Six screening oil degrading bacteria on oil concentration

Isolates code	Oil concentrations (V/V)					
	0.1	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
H26	+	-	-	-	-	-
H27	+	-	-	-	-	-
H28	+	+	+	+	-	-
H29	+	+	+	+	+	-
H30	+	+	+	+	-	-
H31	+	+	+	-	-	-
H32	+	+	+	+	+	+
B33	+	+	+	-	-	-
B34	+	+	+	-	-	-
B35	-	-	-	-	-	-
B36	+	+	+	-	-	-
B37	+	+	-	-	-	-
B38	+	+	+	+	+	-
B39	+	+	-	-	-	-
B40	+	+	-	-	-	-
H41	+	+	+	-	-	-
H42	+	+	-	-	-	-
H43	+	+	+	+	+	+
H44	+	+	+	+	+	-
H45	+	+	-	-	-	-

1. **Phenotypic identification and metabolic fingerprint of the three oil degrading bacterial (ODB) isolates using BIOLOG GEN III:** Recently great attention has been focused on the application of the BIOLOG GEN III system for the identification, confirmation and characterization of over 2900 species of bacteria, yeasts and fungi (Sandle *et al*., 2013). BIOLOG are able to identify the microorganisms at species level and test 94 carbon and chemical sources on a microplate in one shot (El-Liethy, *et al*., 2018).

This system can also give phynotyping metabolic fingerprint and full picture only after incubation period extended from4 to 24h (Park and Rafii, 2014).

In the present study the three oil degrading bacterial isolates that are able to grow on the highest oil concentrations (0.6 (V/V)) were purified and screened several times. The three oil degrading bacterial isolates were identified and the metabolic fingerprints also were determined using BIOLOG GEN III instrument. These strains H17, H32 and H43 were identified as Enterobacter hormaechei, Enterobacter cloacae and Bacillus subtilis, respectively.

The metabolic fingerprints for the three identified were carried out by automated comparing the previously feed data Ominolog system. Figures (4, 5 and 6) illustrate the metabolic fingerprints for the three identified strains, Bacillus subtilius (H43), Enterobacter hormaechei (H17) and Enterobacter cloacae (H32). Enterobacter hormaechei (H17) and Enterobacter cloacae (H32) slightly had the metabolic fingerprints patterns. Both Enterobacter hormaechei (H17) and Enterobacter cloacae (H32) were able to grow and confirmed utilizing the following carbon sources and chemical substrates; Gentiobiose, growth at pH 6, growth at pH 5, D-Sallcin, N-Actyl-β-D-Mannosamine, growth at 1% NaCl, growth at 4% NaCl, D-Galactose, 3 Methyl Glucose, L-Rhamnose, Inosine, 1%Sodium Lactate, Fusidic Acid, Glycerol, D-Glucose 6-PO4, D-Fructose 6-PO4, Troleandomycin, Rifamycin SV, Minocycllne, Glycyl-L-Proline, L-Alanine, L-Aspartic Acid, L-Glutamic Acid, L-Histidine, L-Serine, Lincomycin, Guanidine HCl, Niaproof, D-Galacturonic, D-Gluconic Acid, D-Glucuronic Acid, Mucic Acid, D-Sccric

Acid, Vancomycin, Tetrazollum Violet, Tetrazollum Blue, P-Hydroxy-Phenylacetic Acid, Methyl Pyruvate, L-Lactic Acid, Citric Acid, L-Malic Acid and Acetic Acid.

The only difference in the metabolic fingerprints view between two bacterial strains that, Enterobacter hormaechei (H17) are able to utilize D-serine while and Enterobacter cloacae (H32) not (Table 4 and Figures 4 and 5). Bacillus subtilius (H43) was able to grow and confirmed utilizing the following carbon sources and chemical substrates; Dextrin, D- Maltose, D-Trehalose, D- Cellobiose, Gentiobiose, Sucrose, D- Turanose, able to grow at pH 6 and pH 5, D-Raffinose, B-Methyl-DGlucoside, D-Sallcin, able to grow at 1, 4 and 8% NaCl, D-Fractose, 1%Sodium Lactate, D-Sorbitol, D-Mannitol, Myo-inostol, Glycerol, Gelatin, L-Alanine, L-Aspartic Acid, L-Glutamic Acid, L-Histidine, Pecin, D-Galacturonic, D-Gluconic Acid, Mucic Acid, D-Scchric Acid, L-Lactic Acid, Citric Acid, L-Mallc Acid, Lithium Chloride, Potassium Tellurte, Acetoacetic Acid, and Aztreonam (Table 4 and figure 5).

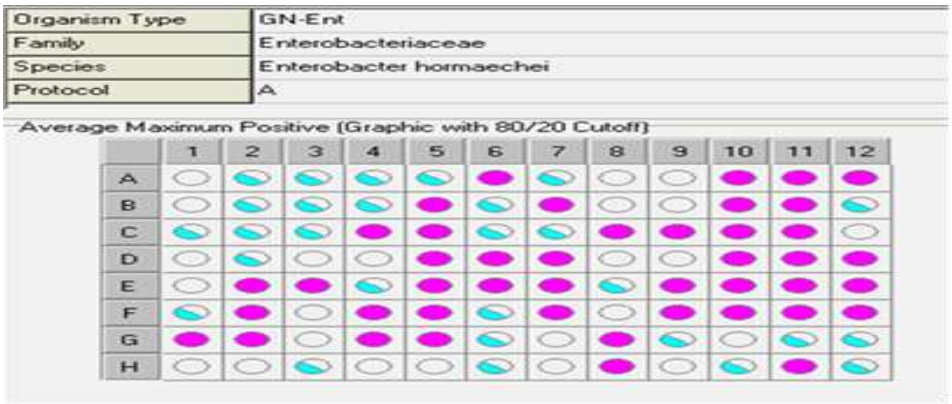


Figure (4): Metabolic fingerprints of Enterobacter hormaechei (H17 strain)

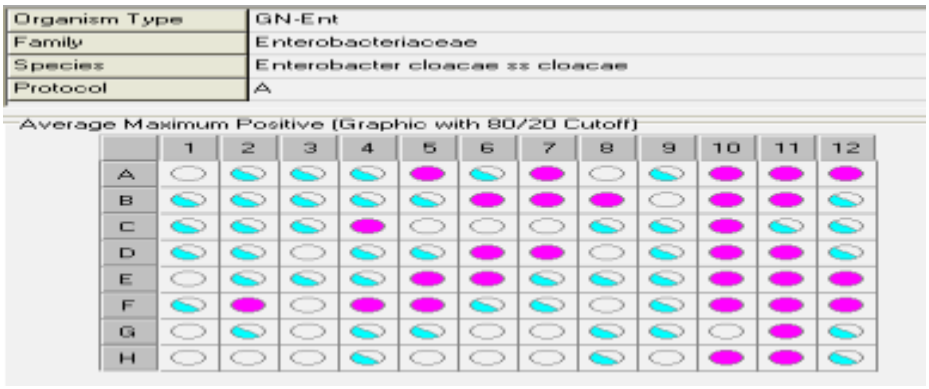


Figure (5): Metabolic fingerprints of Enterobacter cloacae (H32 strain)

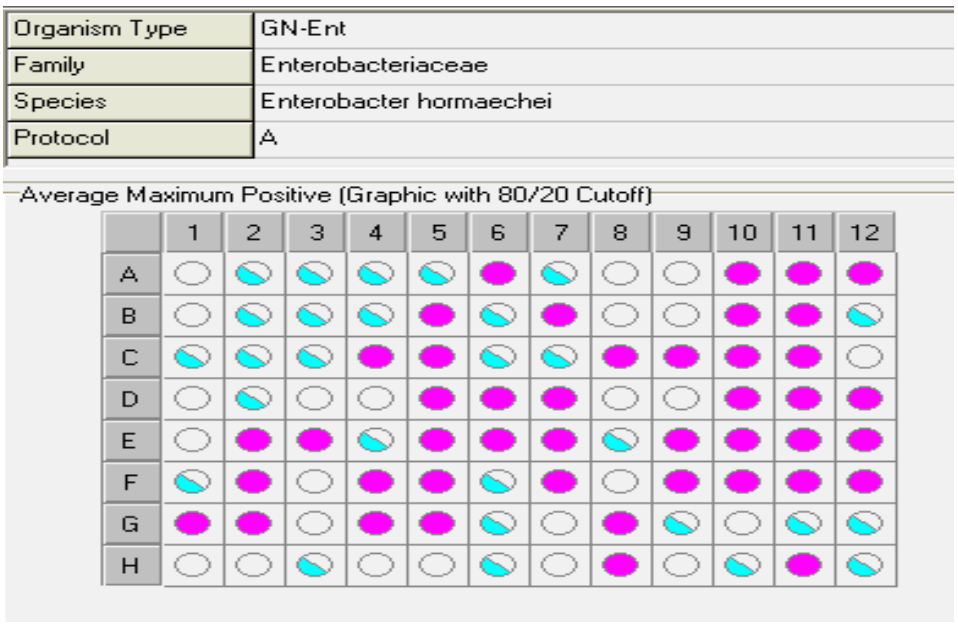


Figure (6): Metabolic fingerprints of Bacillus subtillis (H43 strain)

Table (4): Metabolic fingerprints of the three potent oil degrading bacterial strains using BIOLOG

Properties	Oil degrading bacteria strains			Properties	Oil degrading bacteria strains			Properties	Oil degrading bacteria strains			Properties	Oil degrading bacteria strains		
	H17	H32	H43		H17	H32	H43		H17	H32	H43		H17	H32	H43
Negative control	-	-	-	A-D-Glucose	-/+	-/+	+	Gelatin	-	_	+	P-Hydroxy-Phenylacetic Acid	+	+	-
Dextrin	-/+	-/+	+	D-Mannose	-/+	-/+	_/+	Glycyl-L-Proline	+	+	-/+	Methyl Pyruvate	+	+	-/+
D- Maltose	-/+	-/+	+	D-Fractose	-/+	-/+	+	L-Alanine	+	+	+	D-Lactic Acid Methyl Easter	-	-	-
D-Trehalose	-/+	-/+	+	D-Galactose	+	+	-/+	L-Arginine	-/+	-/+	-/+	L-Lactic Acid	+	+	+
D- Cellobiose	-/+	-/+	+	3 Methyl Glucose	+	+	-	L-Aspartic Acid	+	+	+	Citric Acid	+	+	+
Gentiobiose	+	+	+	D-Fucose	-/+	-/+	-	L-Glutamic Acid	+	+	+	A-Keto-Glutaric Acid	-/+	-/+	-
Sucrose	-/+	-/+	+	L-Fucose	-/+	-/+	-	L-Histidine	+	+	+	D-Malic Acid	-	_	-
D- Turanose	-	-	+	L-Rhamnose	+	+	-/+	L-Pyroglutamic Acid	-	-/+	-/+	L-Malic Acid	+	+	+
Stachyose	-	-	-/+	Inosine	+	+	-/+	L-Serine	+	+	-/+	Bromo-Succinic Acid	-/+	-/+	-/+
Positive control	+	+	+	1%Sodium Lactate	+	+	+	Lincomycin	+	+	-	Nalidixic Acid	-	_	-
pH 6	+	+	+	Fusidic Acid	+	+	-	Guanidine HCl	+	+	-	Lithium Chloride	-/+	-/+	+
pH 5	+	+	+	Serine	-	-	-	Niaproof	+	+	-	Potassium Tellurte	-/+	-/+	+
D-Raffinose	-	-	+	D-Sorbitol	-	-	+	Pecin	-/+	-/+	+	Tween 40	-	_	-/+
A-D-Lactose	-/+	-/+	-	D-Mannitol	-	-/+	+	D-Galacturonic	+	+	+	γ-Amino-Butyric Acid	-	_	-/+

Cont. Table (4): Metabolic fingerprints of the three potent oil degrading bacterial strains using BIOLOG

Properties	Oil degrading bacteria strains			Properties	Oil degrading bacteria strains			Properties	Oil degrading bacteria strains			Properties	Oil degrading bacteria strains		
	H17	H32	H43		H17	H32	H43		H17	H32	H43		H17	H32	H43
D-Mellbiose	-/+	-/+	-/+	D-Arabitol	-	_	-	L-Galactonic Acid Lactone	-	-	-	α-Hydroxy-Butyric Acid	-/+	-/+	-
B-Methyl-DGlucoside	-/+	-/+	+	Myo-inostol	-	-	+	D-Gluconic Acid	+	+	+	β-Hydroxy-D,L-Butyric Acid	-	_	-
D-Sallcin	+	+	+	Glycerol	+	+	+	D-Glucuronic Acid	+	+	-/+	A-Keto-Butyric Acid	-	_	-
N-Acetyl-D-Glucoseamine	-/+	-/+	-/+	D-Glucose 6-PO_4	+	+	-	Glucuronamide	-/+	-/+	-/+	Acetoacetic Acid	-/+	-/+	+
N-Actyl-β-D-Mannosamine	+	+	-	D-Fructose 6-PO_4	+	+	-/+	Mucic Acid	+	+	+	Propionic Acid	-	-	-
N-Actyl-D-Galactosamine	-	-	-	D-Aspartic Acid	-/+	-	-/+	Quinic Acid	-	-	-	Acetic Acid	+	+	-/+
N-Acetyl Neuraminic Acid	-	-	-	D-serine	+	-	-	D-Scchric Acid	+	+	+	Formic Acid	-	-	-/+
1%NaCl	+	+	+	Troleandomycin	+	+	-	Vancomycin	+	+	-	Aztreonam	-/+	-/+	+
4%NaCl	+	+	+	Rifamycin SV	+	+	-	Tetrazollum Violet	+	+	-/+	Sodium Butyrate	+	+	-/+
8% NaCl	-/+	-/+	+	Minocycline	+	+	-	Tetrazollum Blue	+	+	-/+	Sodium Bromate	-/+	-/+	-/+

CONCLUSION

Totally 45 oil degrading bacteria were isolated from oil base mud samples. About 3-90% of total bacterial counts contain oil degrading bacteria. The three potent oil degrading bacteria were identified as Enterobacter hormaechei, Enterobacter cloacae and Bacillus subtilis. The metabolic fingerprints including 94 carbon and chemical sources on a microplate in one shot were carried out as very useful tools for further experimental studies.

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REFERENCES

Al-Joubori, M.H; and Ayad, A. A. H. Abd Al-Razaq; (2008): Biotreatment of Oil Wells Drilling Waste in an Agricultural Soil. I J.chem.pet.Eng. 9, No.2, 69-74.

American Public Health Association (APHA); (2017): Standard Methods for the Examination of Water and Wastewater. 23rd edition, ISBN: 978-0-87553-287-5. Washington D.C.

Atlas, R.M.; (1981): Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective. Microbiol. Rev, 45, No. 1, 180-209.

Balogun, S.A; Shofola, T.C.; Okedeji, A.O. Ayagbenro; P.M.B. (2014): Screening of hydrocarbonoclastic bacteria using redox indicator 2, 6-dichlorophenol indophenol. Global NEST Journal, 17(3); 565-573,

Bourgoyne, A. T.; M. E. Chenevert; K. K. Millheim; and F. S.Young; (2003): Applied Drilling Engineering. vol. 2 of SPE Textbook Series, SPE, Richardson, Tex, USA.

British Petroleum (BP); (2014): Statistical Review of World Energy. Excel workbook of historical data, pp.8-12

Dardira, M.M.; S. Ibrahimea; M. Solimanb; S. D. Desoukya; and A. A. Hafiza; (2014): Preparation and evaluation of some esteramides as synthetic based drilling fluids. Egypt Journal of Petroleum, 23, 1, 35–43.

Das, K. and Mukherjee, A.K; (2007): Crude Petroleum-Oil Biodegradation Efficiency of Bacillus subtilis and Pseudomonas aeruginosa Strains Isolated from A petroleum-Oil Contaminated Soil from North-East India, Bioresour Technol, 98, 1339-1345.

Egyptian General Petroleum Corporation (EGPC); (1992): Western Desert, oil and gas fields (A comprehensive Overview). EGPC, Cairo, Egypt, pp. 431

El-Liethy, M.A.; Hemdan, B.A.; El-Taweel G.E; (2018): Phenotyping using semi-automated BIOLOG and conventional PCR for identification of Bacillus isolated from biofilm of sink drainage pipes, Acta Ecologica Sinica, https://doi.org/10.1016/j.chnaes.2018.01.011

El-Liethy, M.A.; Hemdan, B.A.; Samhan, F.A.; El-Taweel, G.E; (2017): Optimizing conditions for crude oil degrading bacterial consortium isolated from aquatic environment. Poll Res. 36 (2): 197-204.

Energy Information Administration (EIA); (2013): Country Analysis Briefs: Egypt, US Department of Energy, Washington, DC, pp 1-3

Energy Information Administration (EIA); (2015): Country Analysis Briefs: Egypt, US Department of Energy, Washington, DC, pp 1-3

Ferrari, G; Ceccini, F; and Xiao, L; (2000): Drilling wastes treatment and management practices for reducing impact on HSE. ENI/AGIP experiences, Paper SPE 64635 presented at the SPE int. oil and gas conf. and exhibition in China, Beijing. pp: 1-11.

Helmy, Q and Edwan Kardena; (2015): Petroleum Oil and Gas Industry Waste Treatment: Common Practice in Indonesia. J Pet Environ Biotechnol, 6:5

Ijah, U.J.J. and Antai, S.P.; (2003): Removal of Nigerian Light Crude Oil in Soil over a 12- Month Period, Int Biodeterior Biodegradation, 51, 93-99.

Imarhiagbe, E.E and Atuanya, E.I.; (2014): A study of the microbiology and polycyclic aromatic hydrocarbons (PAHs) compositional profile and sources in drill cuttings from Ologbo Oilfield wells at Edo state, Nigeria. SCI WORLD J.9 (1); 8-13.

Luning, P.D. and Pritchard, P.; (2002): Degradation of Polycyclic Aromatic Hydrocarbons Dissolved in Tween 80 Surfactant Solutions by Sphingomonas paucimobilis EPA 505. Can. J. Microbiol. 48; 151-158.

Okpokwasili, G.C. and Okorie, B.B.; (1988): Biodeterioration Potentials of Microorganisms Isolated from Engine Lubricating Oil. Tribol. Inter. 21, 215-217.

Onwukwe S. I. and M. S. Nwakaudu; (2012): Drilling Wastes Generation and Management Approach. INT J ENVIRON SCI TE, 3, pp 3.

Oppenheimer, C. H.; Gunkel, W.; and Gassman, G.; (1977): Microorganisms and Hydrocarbons in the North Sea during July-August 1975, pp. 593-610. In Proceedings of the 1977 Oil Spill Conference. American Petroleum Institute, Washington, DC.

Park, M., Rafii, F. (2014). Global phenotypic characterization of effects of fluoroquinolone resistance selection on the metabolic activities and drug susceptibilities of Clostridium perfringens strains, Intern. J. Microbiol.

Rambeloarisoa, E.; Rontani, J.F.; Giusti, G.; Duvnjak, Z.; and Bertrand, J.C.; (1984): Degradation of crude oil by a mixed population of bacteria isolated from sea surface foams. Mar Biol. 83: 69-81.

Sandle, T., Skinner, K., Sandle, J. Gebala, B., Kothandaraman, P. (2013). Evaluationof the GEN III OmniLog® ID System microbial identification system for the profiling of cleanroom bacteria, Eur J Pharm Sci.18 (2): 44–50.

Sun, Q., Bai, Y., Zhao, C., Xiao, Y., Wen, D. and Tang, X. (2009): Aerobic biodegradation characteristics and metabolic products of quinoline by a Pseudomonas strain. Bioresour Technol, 100: 5030-5036.

Williams, S.T. and Gray, T.R.G.; (1973): General Principles and Problems of Soil Sampling. Microbiological Monitoring of Environments, Academic Press, London, pp 66-110.

World Bank database (WBD); (2015): Accessed May 2015.

Xia, X.H.; Yu, H.; Yang, Z.F.; and Huang, G.H.; (2006): Biodegradation of Polycyclic Aromatic Hydrocarbons in the Natural Waters of the Yellow River: Effects of High Sediment Content on Biodegradation. Chemosphere, 65; 457-466.

Youssef, M.; (2010): Hydrocarbons Utilizing Bacteria - Distribution and Biodegradation Potentials - in Ismailia Canal. Master thesis, Microbiology Department, Faculty of Science, Al-Azhar University (Girls), Cairo, Egypt.

Zhang, G.; Yue-ting, W.; Xin-ping, Q.; Qin, M.; (2005): Biodegradation of crude oil by Pseudomonas aeruginosa in the presence of rhamnolipids. J Zhejiang Univ Sci B. 6B (8): 725-730.

عزل و توصيف البكتريا المستهلكة للزيت والمعزولة من مخلفات طين الحفر ذو القاعدة الزيتية فى الصحراء الغربية المصرية

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المستخلص

تهدف هذه الدراسة إلى عزل وفحص وتعريف أقوى أنواع البكتريا المحللة للزيت والمعزولة من مخلفات طين الحفر الزيتى الناتج عن مجموعة مختارة من آبار التنقيب عن النفط والغاز واستكشاف الآبار. بالإضافه إلى الهدف الآخر لهذه الدراسة هو تحديد البصمة الأيضية لأقوى بكتريا محللة للزيت والتى تم عزلها من نفس العينات. ولتحقيق هذه الأهداف، فقد تم تجميع ١١ عينة من مخلفات طين الحفر ذو القاعدة الزيتية من مواقع حفر مختلفة في الصحراء الغربية، مصر، كما تم تحديد

أعداد البكتيريا الكلية عند درجتى حرارة هى ٢٢ و ٣٠ درجة مئوية لإعطاء صورة كاملة عن وجود كل أنواع البكتيرية والموجوده بصوره طبيعية في العينات التي تم جمعها. علاوة على ذلك ، فقد تم تحديد أنواع البكتريا المحللة للزيت على نيئة أجار الزيت المحتوية على ٠,١٪ من الزيت (V / V) باستخدام طريقة الفرد السطحى على الاجار. ولقد أظهرت النتائج أن متوسط أعداد البكتيريا الكليه عند درجتى حراره ٢٢ و ٣٠ درجة مئوية هى (1.4x 104) و (1.6x 104) خلية بكتيريه لكل جرام، على التوالي. علاوة على ذلك، فقد تراوحت أعداد البكتريا المحللة للزيت ما بين(1.0 x 102) إلى (6.1 x 103) خلية بكتيريه لكل جرام طين في جميع العينات المجمعة. وقد تم أيضا تحديد أقوى أنواع البكتيريا المستهلكة للزيت وذلك باستخدام جهاز البيولوج على النحو التالي Enterobacter hormaechei، Enterobacter cloacae و Bacillus subtilis. ولقد وجد أن الفرق الوحيد في بصمات الأيض هو أن Enterobacter hormaechei (H17) قادرون على استخدام D–serine فى حين أن Enterobacter cloacae (H32) لا يمكنها ذلك. يمكن الاستنتاج أن بصمات الأيض الخاصة بأكثر أنواع البكتريا المستهلكة للزيت توفر معلومات كاملة عن أنشطة التمثيل الغذائي الميكروبي التي قد تكون مفيدة في تحديد الظروف الملائمة التي تساعد في تجارب التحلل البيولوجي.

كلمات وعبارات داله: الطين ذو القاعدة الزيتية، مخلفات الحفر، البكتيريا المكسرة للزيت، الصحراء الغربية، مصر.