Isolation and Molecular Characterization of Novel Bacterial Strains Possessing High Efficiency to Degrade Mineral Oil

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Abstract: Bacteria possessing high capacity to degrade mineral oil, were isolated, identified and screened. Among isolates, six strains, BGERC 3, BGERC 6, BGERC 13, BGERC 10, BGERC 14, and BGERC 9 were identified using sequencing of 16S-rRNA gene as new strains of *Achromobacterspanius*, *Achromobacterxylosoxidans, Bacillus subtilis, Achromobacterfusiformis, Pseudomonas aeruginosa and Ochrobactrumanthropi* species, respectively, showed relatively high capacity and wide spectrum to degrade the hydrocarbons in mineral oil. All strains showed positive response up to 98.6% when grown on 1, 3, and 5% hydrocarbon by the fifth day. Fingerprinting and assessment of genetic variability of isolated strains were carried out via RAPD-PCR technique using ten RAPD primers. High polymorphism level was detected (90.4%). The highest genetic similarity was between BGERC3 and BGERC 6 (35.3 %), while the genetic similarity between BGERC10 and BGERC13 was the lowest (11.6%). The mean of genetic similarity among the six bacterial isolates was 0.0.19. The bacterial strains BGERC6, BGERC 13, and BGERC 14 were two fold more biodegradation activity of hydrocarbon than strains BGERC 3, BGERC 9, and BGERC 10. About 94.6, –98.6% of excess amount of total added mineral oil to mineral salts media as a sole carbon source could be degraded by BGERC 6, BGERC 13, and BGERC 14 within 5 days. Thus, these three isolates have potential to be useful for bioremediation of sites highly contaminated with petroleum hydrocarbons. The two genera of *Achromobacter* and *Ochrobactrum* are first to isolate and report in bioremediation petroleum hydrocarbon studies.
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Key words: Bioremediation, hydrocarbon, bacterial isolates, 16S-rRNA gene, genetic fingerprinting.

1. Introduction

The class of petroleum products known as mineral oils can be generally understood to include a variety of products which go by different names such as white oils, lubricating oils, light fuel oils, residual fuel oils, as well as transformer and cable oils (Gary and Handwerk, 2001).Mineral oils refer to all oils which are made from dewaxed paraffin-based crude oils which are blended with additives to particular properties for specific uses (Aluyorand Ori-jesu, 2009).Mineral oils are composed of straight and branched chain paraffinic, naphthenic, and aromatic hydrocarbons with 15 or more carbons in a complex mixture (Aluyor and Ori-jesu, 2009).

The uses of mineral oils includes applications such as engine oils, automotive and industrial gear oils, transmission fluids, hydraulic fluids, circulating and hydraulic oils, bearing oils and machine oils. Other uses are as machine-tool oils, compressor and refrigerator oils, textile machine oils, air tool oils, steam engine oils, metalworking oils, rust prevention oils transformer oils and so on and so forth (Aluyor and Ori-jesu 2009). The lubricant oil discarded in the nature is a cause of concern due to a non-quantified impact, because of its potential chronic damage to the human health. It is impossible to avoid totally the emission of this effluent directly in the environment (Wright *et al.,* 1993), and the impact caused a decreasing of microbiota biodiversity (Atlas *et al.,*1991).

The mineral oils such as lubricant oil could persist in the environment for more than six years in someecosystems, causing chronic problems for the biota (Burns *et al.,*1994). The biodegradability is the most important aspect when the substance is discarded in the environment (Eisentraeger*et al.,*2002).

Bioremediation (Microbial degradation) has been shown to be a potent technique for the breakdown of contaminations into less harmful form (Pratt *et al.,*1999). To attenuate the environmental effect caused by water pollution by hydrocarbons derived from mineral oil, the bioremediation was less aggressive and more adjusted to maintain the ecological balance (Rosato, 1997).

Microbial remediation of hydrocarbon compounds was found to be an available alternative method over the conventional methods. Microbial

treatment can control hydrocarbons pollution by reducing the length of the hydrocarbon molecules and by producing by-products that act as biosurfactants and solvents (Banat, 1995 and Wolicka*et al.,*2009).

An enabling environment facilitates the degradation of mineral oil by microorganisms; pH, temperature and other growth factors required by the organisms should be optimal. Microbial degradation of mineral oil and its derivatives is an important applicationof biotechnology research because of the impact of oil spills in the environment. Information about biodegradation of mineral oil in Arabian Gulf area is scarce.

Various molecular techniques have been developed which permit species identification and typing microorganisms, including Bacteria.Randomly amplified polymorphic DNA (RAPD) analysis seemed to be efficient in distinguishing different isolates; it has a high discriminatory power, it is easy to perform, does not require radiolabelled probes, and it is applicable to several microorganisms (Robert *et al.,*1995). It can be used to reliably type yeast strains (Baleiras*et al.,*1995, 1996; Molnár*et al.,*1995).

An excellent target for bacterial identification and phylogenetic characterization is 16S-rRNA gene, the 16S-rRNA gene is universally distributed and highly conserved (Woese, 1987). Due to itsconserved nature and ease of manipulation, it has been extensively used to establish accurate identification of bacterial isolates.

Over the last few years, the increasing use of PCR, rapid template purification, and automated DNA sequencing has dramatically reduced the time necessary to yield a high-quality sequence. The use of 16S-rRNA gene sequencing to study the relatedness of prokaryotic species is well established and has led to increased availability of 16S-rRNA databases. The convergence of these technical and computational advances has also enhanced the application of 16S-rRNA gene sequence analysis to bacterial identification (Rantakokko-Jalava*et al.,*2000). It was recently reported that subtle sequence differences in the 16S rRNA gene could be used for species identification (Sacchi*et al.,*2002) and for subtyping and identifying hypervirulent bacterial clones (Nilsson *et al.,*2003).

The present study aims to isolate, characterize, fingerprintnew bacterial isolates able to degrade mineral oil.

2. Materials and Methods

Isolation of microorganisms

The microorganisms used in this study were obtained by the enrichment culture technique by inoculating Bushnell- Haas enrichment mineral medium containing (g/l): $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1.0; KH_2PO_4, 1.0; $FeCl_3$, 0.05; NH_4NO_3, 1.0; $CaCl_2$, 0.02; pH to 7.2 and sterilize at 121 ^oC for 15 min with soil polluted with crude oil. Bacteria were grown in 250 ml Erlenmeyer flasks for two months in a rotary shaker for at least 20 cycles. Flasks were amended with crude oil 1, 2, 4, and 6 mL crude oil 100 mL^{-1} (Al-Ghawar field) in eastern of KSA.

Identification microbial strains

The morphological characteristics of the isolates were identified by gram stain and biochemical reactions. The biochemical reactions include glucose fermentation, oxidase test, catalase production reaction, cell motility and reactionintryptose soya broth were performed. According to Bergey's Manual of Determinative Bacteriology (Holt *et al.,*1994) and the APi Kit profiling (Api, bioMerieux, France, 2009) isolates were isolated, identified and named based on morphological, physiological, biochemical characteristics and 16S-rRNA gene.

Culture growth conditions

Bushnell- Haas enrichment mineral media were used. The media were amended with mineral oil 1, 3 and 5% (v/v) for each isolate. The pH of mediawas adjusted to 7. One ml was taken to measure turbidity at 595 nm with spectrophotometer.

Optical density and biomass measurement

The turbidity of the cultures was determined by measuring the Optical Density (OD) at a wavelength of 595 nm in 2 ml cuvettes using a spectrophotometer (Biophotometer plus, Eppendorf). The net dry weight for the biomass was determined simultaneously. A 1 mL of culture was centrifuged at 1500 rpm for 10 min, washed twice with distilled water, poured into a pre-weighed container, dried overnight at 90 °C to constant weight and cooled for reweighing. The linear relation between $O.D_{595}$ and dry mass was obtained. Cultures were usually harvested at absorbency 0.660. Cell numbers will be no longer linear with respect to absorbency above this value. Also, pH of the medium should not change when experiments was terminated at this absorbency. Cells were harvested by centrifugation for 5 min at 3,000 x g at room temperature.

Growth rate measurement

The growth rates of cultures in exponential phase were determined from linear regressions of log10 absorbency vs. time, calculating a least squares fit of data from the exponential growth phase, and determining the slope of this line. The instantaneous growth rate (μ) was determined from the slope of this line x ln10; μ hadthe dimensions h^{-1} (Koch, 1981).

Hydrocarbon measurements

Samples taken from the flasks were mixed with equal volumes of hexane and shaken to extract mineral oil. Residual mineral oil was determined using the method used by Martinez-Checa*et al.*(2002).

Molecular genetics analysis

DNA Extraction:

The cell pellets form all isolates were used to extract genomic DNA using (Jena Bioscience, Germany) extraction kit according to manufacturer's instructions.

Random amplified polymorphic DNA (RAPD)

Ten different primers were used in PCR reaction which consists of 10*ρ*mol of each arbitrary 10-mer primers and 25 to 50 *n*g of genomic DNA and 12.5 µl of 2x SuperHot PCR Master Mix (Bioron, Ludwigshafen, Germany). The codes and sequences of these oligoprimers are listed in Table (3). The RAPD-PCR amplification reactions were performed in Eppendorf® thermal cycler using the following PCR program: 1cycle at 94°C, 4 min; 35 additional cycles consisting of 94°C 5 s, 37°C 20 s and 72°C 20 s. After the amplification, the PCR reaction products were electrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 x 14 cm 1.5%agarose gel (Bioshop, Canada) for 30 min using Tris-borate-EDTA Buffer. The gel was stained with 0.5 µg/ml of ethidium bromide (Bioshop, Canada).

PCR amplification of 16S-rRNA gene

Primer sequences used to amplify the 16S-rRNA gene fragment were: U1 [5CCA GCA GCC GCG GTA ATA CG3] and U2 [5ATC GG(C/T)TAC CTT GTT ACG ACT TC3] according to Kumar et al., (2006).The PCR master mix contained10 Pmol of each primer and 12.5 µl of 2xSuperHot PCR Master Mix (Bioron, Ludwigshafen, Germany) mixed with 50 to 100 ng of DNA template. Sterile d.H2O was added to a final volume of 25 µl. Thermal cycler (Uno II , Biometra, Germany) program was 94 °C for 4 min., 94 °C for 1 min., 55 °C for 1 min., 72 °C for 1.5 min, the number of cycles was 35 cycle and the post PCR reaction time was 72°C for 5 min.

Analysis of the PCR products

After the amplification, the PCR reaction products were electrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 x 14 cm 1.5%-agarose gel (Bioshop, Canada) for 30 min using Tris-borate- EDTA Buffer. The gels were stained with 0.5ug/ml of ethidium bromide (Bioshop, Canada), visualized under the UV light and documented using a GeneSnap 4.00- Gene Genius Bio Imaging System (Syngene, Frederick, Maryland, USA).

Gels analysis

The agarose digital image files were analyzed using Gene Tools software from Syngene. The densitometric scanning of each based on its three characteristic dimensions was carried out. Each band was recognized by its length, width and intensity. Accordingly, the relative amount of each band was measured and scored.

Sequencing of 16S-rRNA gene

The 990bp PCR-products of each isolate were purified from excess primers and nucleotides by the use of AxyPrep PCR Clean-up kit (AXYGEN Biosciences, Union City, California,USA) and directly sequenced using the same primers as described for the amplification process. The products were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI Applied Biosystems, Foster City, California, USA) on a 3130XL Genetic Analyzer (Applied Biosystems). The bacterial 16S-rDNA sequences obtained were then aligned with known 16S-rDNA sequences in Genbank using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information, and percent homology scores were generated to identify bacteria.

Determination of Genetic relationship

In order to determine genetic relationship among studied bacteria RAPD bands were scored for presence (1) or absence (0). The data were transferred to a statistical software program, Statistical Package for Social Science (SPSS), version 10 (SPSS Inc., Chicago, Illinois, USA) to obtain statistical analysisin the form of Jaccard's similarity coefficient (S) showing the genetic similarity among different examined bacterial isolates based on pair-wise comparison. The dendrogram was constructed using the Average Linkage between groups.

3. Results

Isolation of oil biodegrading bacteria

Thirty one microbial isolates representing the different colony morphologies observed with the ability to grow on mineral oil as sole carbon and energy source were obtained. Six isolates were selected for further work according to good growth on mineral media amended with mineral oil hydrocarbon as sole carbon and energy source (Table 1).

Identification of isolates

The bacterial isolates were subjected to morphological and biochemical tests (Table.1). The isolates possessed typical cellular and colonial morphologies, physiological, biochemical, and nutritional features that resembled it to four different genera *Achromobacter,Ochrobactrum , Pseudomonas and Bacillus*. In this study two genera were dominant

isolates*Achromobacterand Ochrobactrum.* Furthermore, *Bacillus fusiformis species* was repeatedly isolated.The two genera of *Achromobacter* and *Ochrobactrum* are first to isolate and report in bioremediation petroleum hydrocarbon studies.

Growth rates on mineral oil hydrocarbon

Growth curve of isolates (Fig. 1 and 2) on Bushnel-Haas mineral medium amended with 1% mineral oil hydrocarbon showed different specific growth rates (Table 2). The specific growth rates of the different isolates on mineral oil showed isolates BGEC 3, BGEC 10 and BGEC 13 to be the fastest growing in mineral salts medium containing 1 % (v/v) mineral oil. Most growth occurred in the first 5 and 6 days for all isolates resulting in good biomass production (Fig. 1 and 2). Maximum specific growth rates (μ_{max}) for BGEC 6 and BGEC 10 isolates were after two and three days of growth being 0.163 h^{-1} and 0.167 h^{-1}, respectively. Strains were also grown on 3% and 5% hydrocarbon (data not shown). However, growth on 1% hydrocarbon was better than 3 and 5 % (v/v) concentrations. No change was observed in pH during the first seven days of incubation for all strains(data not shown).

The growth rate (μ_m) of 0.163, 0.203 and 0.341h^{-1} was recorded for isolates BEGRC 6, BEGRC 13, and BEGRC14 compared with only 0.288, 0.312 and 0.137 h^{-1} for isolates BEGRC 3, BEGRC9 and BEGRC 10, respectively (Table 2). Moreover, isolate BGEC 3 produced more biomass from hydrocarbons being 0.052 g cells l^{-1}mineral oil hydrocarbon. The biomass values recorded for the five isolates were 0.04, 0.041, 0.39, 0.049 and 0.045gcell/g mineral oil hydrocarbon after five days of growth, respectively (Fig. 1 and 2).

Biodegradation of mineral oil hydrocarbon

The activity of six strains on 1,3, and 5 % (v/v) mineral oil was monitored and the growth rate, biomass yield, and rate of degradation were used as indication for the ability of these isolates to grow on petroleum hydrocarbon. All strains showed positive response up to 98.6% when grown on 1, 3, and 5% hydrocarbon by the fifth day. The bacterial isolate BGERC6, BGERC13, and BGERC14 were two fold more biodegradation activity of hydrocarbon than strains BGERC3, BGERC9, and BGERC10 (Fig. 3). The lowest activity was recorded on 5% mineral oil. The biodegradation of hydrocarbon was starting to decrease after the fifth day. The higher the concentration of mineral oil hydrocarbon the lower of biodegradation activity.

Genetic identification and Molecular characterization

Sequencing of 16S- rRNA gene and RAPD as a PCR based techniques were used to identify the selected bacterial isolates. In addition conducting of genetic fingerprinting, constructing genetic relationship and detecting specific molecular markers for most potent isolate were accomplished.

According to the alignment at the National Center for Biotechnology Information (NCBI), the studied isolates BEGC 3, BEGC 6, BEGC 9, BEGC10, BEGC 13and BEGC 14were identified as *AchromobacterspaniusAchromobacterxylosoxidans, Bacillus subtilis, Bacillus fusiformis, Pseudomonas aeruginosa* and *Ochrobactrum anthropic,* respectively. Based on the RAPD results, ten primers showed successful PCR amplification. An informative profile was obtained (Figure 4). These primers resulted in produce 351 PCR bands among 178 amplified bands (Table 3). Out of which 161 and 17 bands were polymorphic (90.4%) and monomorphic (10.6) respectively. The ten primers produced multiple band profiles with a number of amplified DNA fragments ranging from 8 to 24. The size and number of amplified fragments also varied from 280bp to 1800bp with different primers. The maximum number (48 fragments) was amplified with (OPC-6, OPC-7 and OPC-8) primers and the minimum number (14 fragments) was amplified with primer (OPC-1). Results illustrated in (Table 4) and represented in Fig. (5) demonstrated the genetic relationships among selected six isolates based on data recorded from polymorphism across RAPD markers.

The dendrogram showed Phylogenetic tree which divided into two clusters. The *Achromobacterspanius* (BGEC13) formed a distinct isolate, separated from all of the others studied isolates. The first cluster includes three bacterial isolates (BGEC3, BGEC6 andBGEC9). In this cluster, the lowest genetic distance was detected between BGEC3 and BGEC6isolates, but the isolate BGEC9 was closely related. The second cluster consisted of two bacterial isolates (BGEC10 and BGEC14) with average of similarity coefficient 0.196.

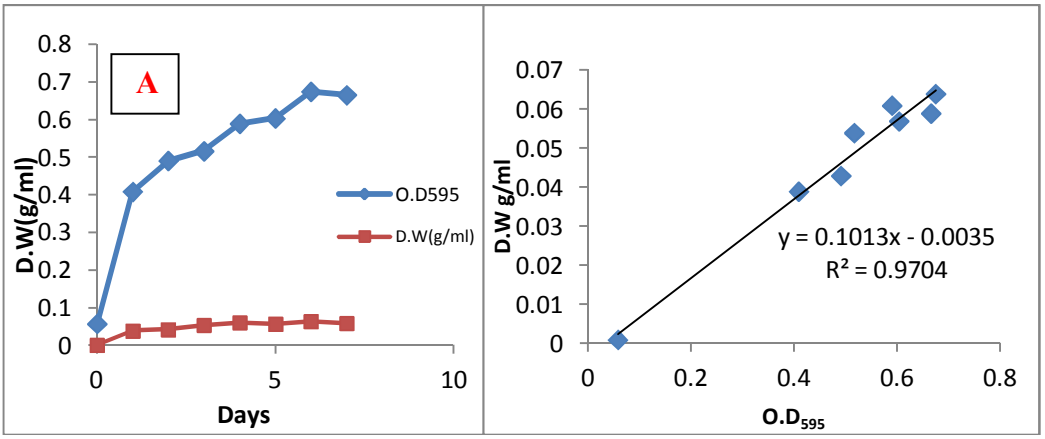
The genetic similarity was calculated and illustrated in Table 4. The highest genetic similarity was between BGEC3 and BGEC6 (35.3 %), while the genetic similarity between BGEC10and BGEC13 was the lowest (11.6%). The mean of genetic similarity among the six bacterial isolates was 0.19.

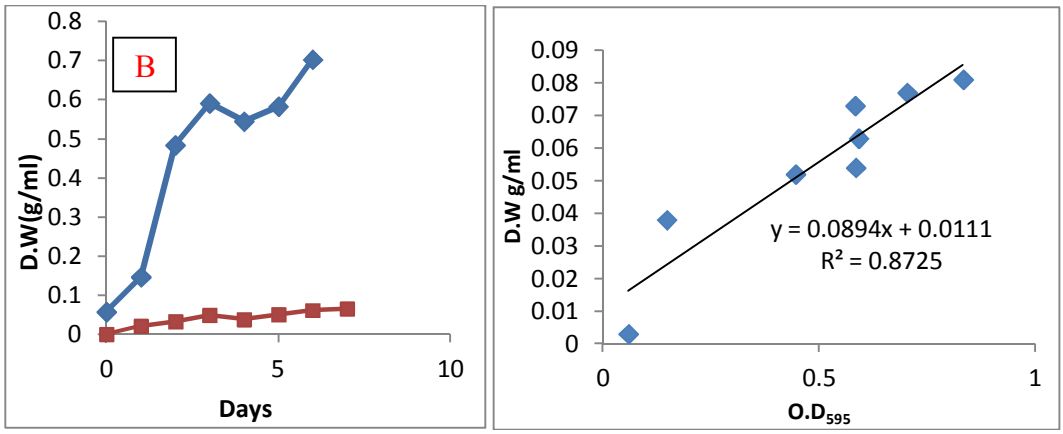
Table 1: Morphology, physiology, and growth of biodegrading bacteria.

Identification Code	BEGC3	BEGC6	BEGC9	BEGC10	BEGC13	BEGC14
Proposed Name	*Achromobacterxylosoxidans*	*Pseudomonas aeruginosa*	*Ochrobactrum anthropi*	*Bacillus subtilis*	*Achromobacter spanius*	*Bacillus fusiformis*
Colony color	Greenish	Greenish	Creamy	Yellow	Creamy	Creamy
Morphology	*Bacilli*	Short rods	Short rods	*Bacilli*	Short rods	*Bacilli*
Gram Stain	-	-	-	+	-	+
Motility	+	+	-	+	+	+
Oxidase reaction	+	+	+	+	+	+
Catalase reaction	+	+	+	+	+	+

Table 2. Growth rates (μ_m) of isolated strains growing on Bushnell- Haas mineral medium amended with 1% hydrocarbon.

Strain	Growth rate (μ_m) h^{-1}
*Achromobacterxylosoxidans*BEGC 3	0.288
*Pseudomonas aeruginosa*BEGC 6	0.163
*Ochrobactrumanthropi*BEGC 9	0.312
*Bacillus subtilis*BEGC10	0.137
*Achromobacterspanius*BEGC 13	0.203
*Bacillus fusiformis*BEGC 14	0.341





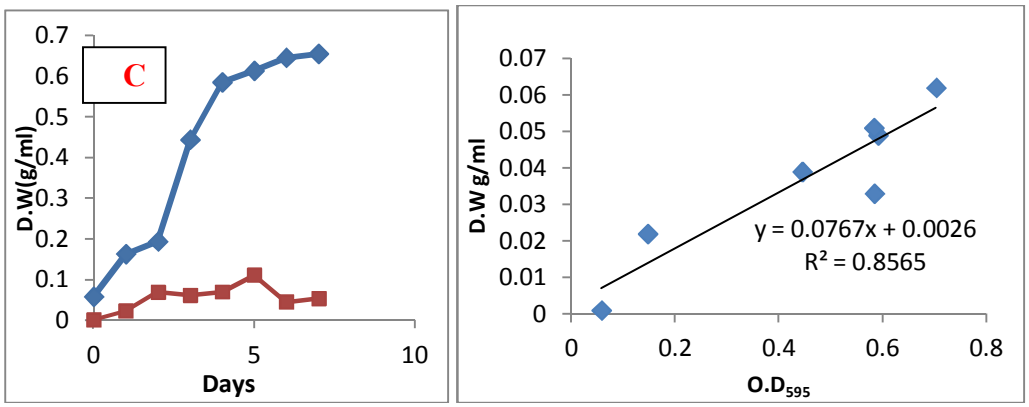


Fig.1. Growth curve of strain *Achromobacterxylosixidans* (A), *Pseudomonas aeruginosa* (B), and *Ochrobacteriumanthropi* (C), on 1% (v/v) of mineral oil hydrocarbon concentrations for 5 days.

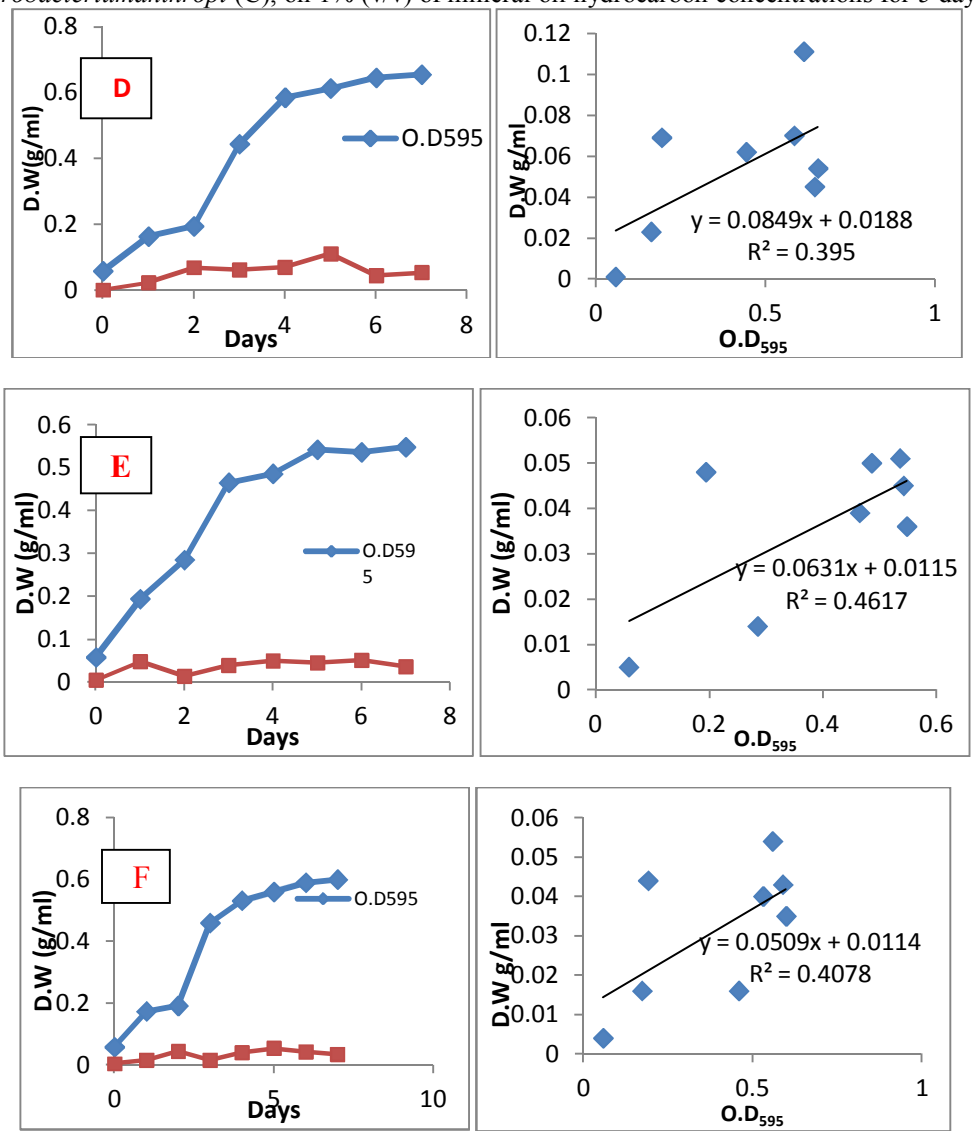


Fig.2. Growth curve of strain *Bacillus subtilis* (D), *Achromobacterspanius* (E), and *Basillusfusiformis* (F) on 1% (v/v) of mineral oil hydrocarbon concentrations for 5 days.

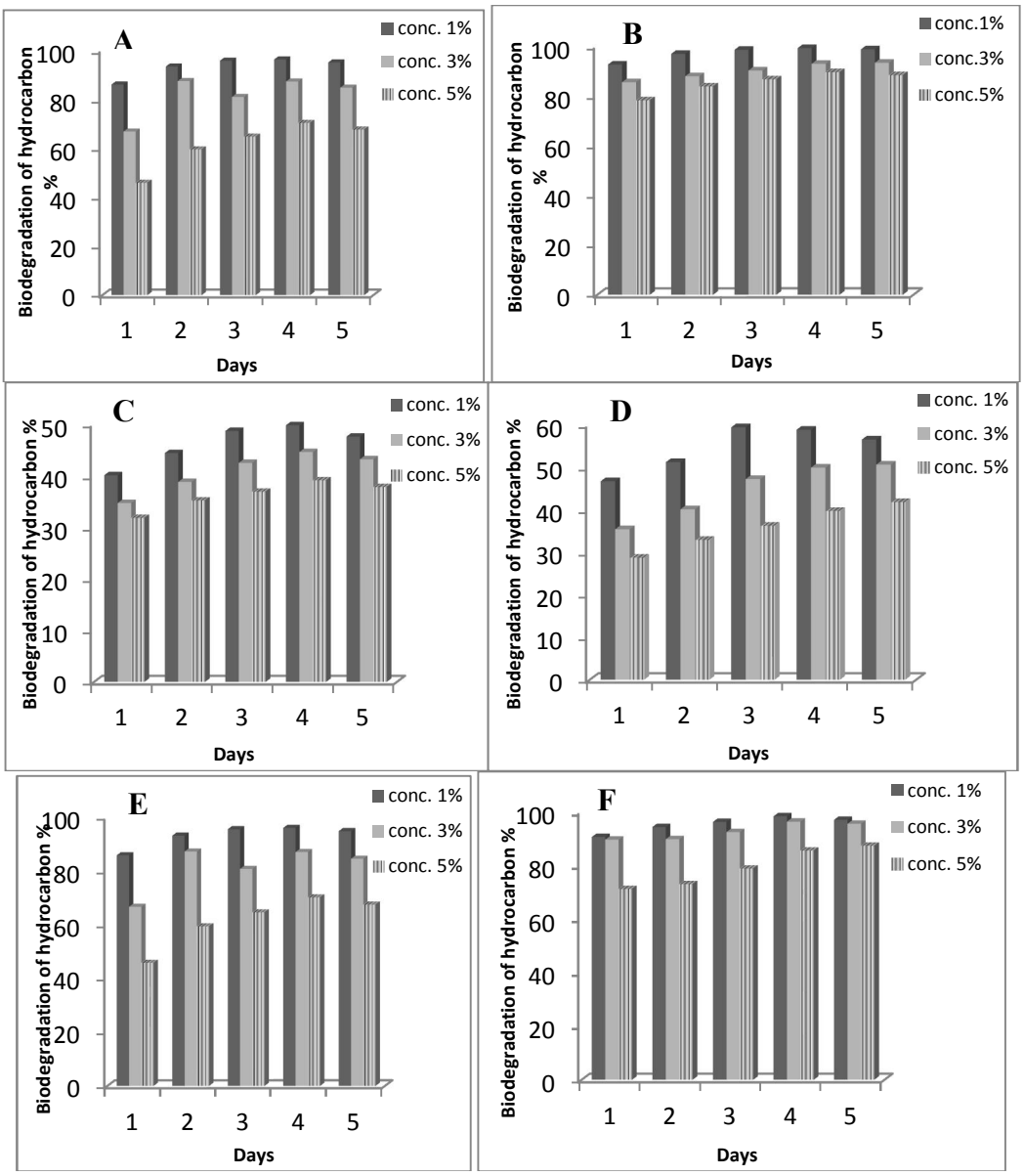


Fig.3. Biodegradation of *Achromobacterxylosixidans* (A), *Pseudomonas aeruginosa* (B), *Ochrobacteriumanthropi* (C), *Bacillus subtilis* (D), *Achromobacterspanius* (E), and *Basillusfusiformis* (F) on different hydrocarbon concentrations of mineral oil for 5 days**.**

Table3.List of primers, nucleotide sequences, total number of bands, monomorphic and polymorphic bands produced by ten primers among six bacterial isolates.

Primer code	Prime sequence	Isolates*						Total bands	Amplified bands	Monomorphic bands	Polymorphicbands
		3	6	9	10	13	14				
OP-C6	5'-GAACGGACTC-3'	12	6	11	7	5	7	48	**24**	**2**	**22**
OP-C1	5'-TTCGAGCCAG -3'	2	2	1	3	1	5	14	**8**	**0**	**8**
OP-C7	5'-GTCCCGACGA-3'	12	5	12	2	8	9	48	**22**	**1**	**21**
OP-C8	5'-TGGACCGGTG-3'	8	10	9	5	8	8	48	**20**	**4**	**16**
OP-C9	5'-CTCACCGTCC-3'	7	7	7	1	6	11	39	**19**	**1**	**18**
OP-C10	5'-TGTCTGGGTG-3'	8	3	7	8	7	7	40	**20**	**4**	**16**
OP-D4	5'-TCTGGTGAGG-3'	3	0	2	2	3	7	17	**12**	**0**	**12**
OP-D6	5'-ACCTGAACGG-3'	0	5	9	9	5	3	31	**18**	**1**	**17**
OP-D7	5'-TTGGCACGGG-3'	5	7	6	5	6	7	36	**15**	**2**	**13**
OP-D8	5'-GTGTGCCCCA-3'	5	8	8	6	9	4	40	**20**	**2**	**18**
total		62	53	72	48	58	68	**361**	**178**	**17**	**161**

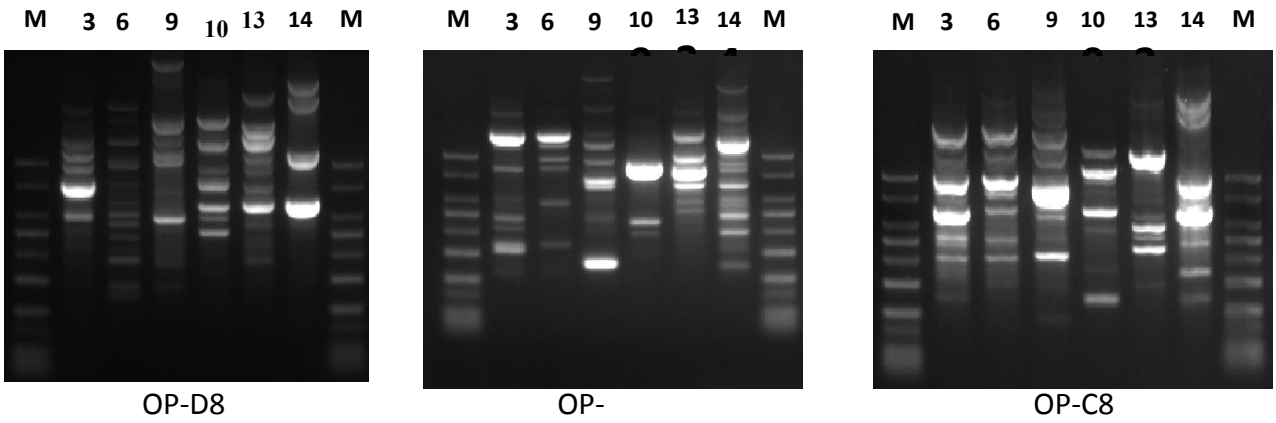


Fig.4. RAPD fingerprinting patterns of six bacterial isolates with OP-D8, OP-C9 and OP-C8 primers.

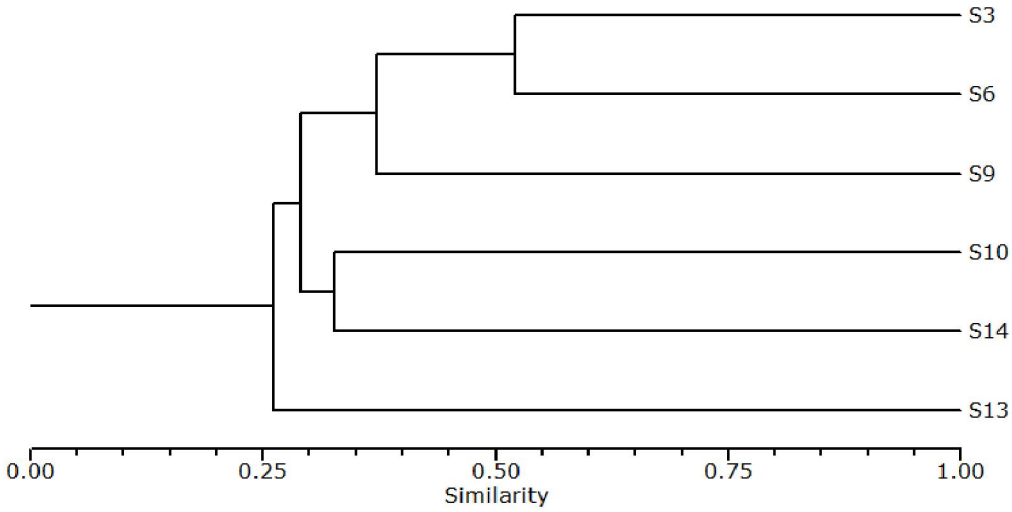


Fig.5. Phylogenetic tree of six bacterial isolates based on their RAPD fingerprints

Table (4): Similarity coefficients among the studied bacterial strains.

	BEGC 3	BEGC 6	BEGC 9	BEGC10	BEGC 13	BEGC 14
BEGC 3	1.00					
BEGC 6	0.52	1.00				
BEGC 9	0.33	0.42	1.00			
BEGC10	0.27	0.24	0.27	1.00		
BEGC 13	0.30	0.27	0.28	0.21	1.00	
BEGC 14	0.31	0.35	0.31	0.33	0.25	1.00

4. Discussion

Biodegradation of mineral oil, agricultural agrochemicals, and other environmental pollutants in natural ecosystems is quite complex, as it occurs relatively slow. Soil contaminated with mineral oil has posed a great hazard for terrestrial and marine ecosystems. So far, biodegradation suggests an effective method (Morgan and Watkinson, 1989). During biodegradation, mineral oil is used as an organic carbon source by a microbial process, resulting in the breakdown of mineral oil components to low molecular weight compounds. Mineral oil, water-in-oil emulsion, and rhydraulic fluids all have components in common with a very large number of other products that are based on mineral oil and synthetic mineral oils (polyalphaolefins).

The isolates belong to the following genera and species:*Achromobacterxylosoxidans, Pseudomonas aeruginosa, Ochrobactrumanthropi, Bacillus subtilis, Achromobacterspanius*and*Bacillus fusiformis*.So, high polymorphism was observed (90.4%).

The results obtained by bacteriology identification techniques were in agreement with those obtained from PCR-based sequencing 16s-rRNA gene technique. RAPD-PCR is a genotypic identification and characterization system that has shown great specificity and sensitivity to define bacterial isolates. This system uses random primers under low specificity conditions; it is less costly and faster and easier to perform than analogous systems, either phenotypic or genotypic (Welsh and McCleland 1990) and (Williams *et al.,* 1990). RAPD-PCR yield different information, since they analyze different sequences and detect different types of variations in the bacterial DNA. RAPD-PCR detects differences along the entire bacterial genome, not only in particular sequences. Thus, this system is helpful in characterizing bacterial isolates over long periods (Bukanov *et al.,*2003) and Struelen *et al.,*1993).

RAPD-PCR products revealed high level of genetic variability which explored by different size and number of amplified PCR bands. These results indicated that high level of genetic heterogeneity among studied six bacterial isolates. Moreover, the used primers in the present investigation proved to be quite powerful in distinguishing different isolates (Kamaleldin *et al.,*2003). Low similarity coefficient average (0.21) is considered as another evidence for high level of genetic variability between studied isolates.

Studies have shown that lower doses of mineral oil were more highly utilized than higher doses (Etoumi, 2007). This could account for the high growth response in the low dose of mineral oil (1%). The result is consistent with that of Lizarraga-Partida *et al.*(1982), who observed that mineral oil has little or no effect on the total heterotrophic bacteria of an environment. The presence of certain microorganisms in both locations shows the interrelationship of the microorganisms in the complex environment. In fact, when bioremediating mineral oil occurred, the following should be considered. Firstly, the oxygen is required because biodegradative pathways are aerobic processes. Secondly, many microorganisms are capable of aliphatic hydrocarbons degradation. Thirdly, soil normally contains an adequate inoculum of natural organisms for bioremediation. Indigenous soil microbes may be too efficient in breaking down chemicals, acting before pesticides have had a chance to protect crops. *Bacillussubtilis*, *Psedomonasaerogenosa* and *Halomonaseurihalina* species were effective bacteria in the biodegradation of mineral oil hydrocarbon (Martinez-Checa *et al.,*2002, Sadeghazad and Ghaemi, 2003 and Shahaby and El-Tarras, 2011).

Microbial populations that consist of strains that belong to various genera have been detected in petroleum-contaminated soil or water (Sorkhoh *et al.,*1995, Chikere *et al.,*2009). This strongly suggests that each strain or genera have their roles in the hydrocarbon transformation processes.The isolation of new pure strains from such a study has alsobeen achieved, its mineral oil degradation ability confirmed, and thedifferent effects of mineral oil on their degrading capacity have been shown.The isolates possessed typical cellular and colonial morphologies, physiological, biochemical, and nutritional features that resembled it to four different genera:*Ochrobactrum* spp., *Bacillus*spp**.,** *Pseudomonas spp, and Achromobacter*spp.Thus, these three isolatesBEGRC 6, BEGRC 13, and BEGRC14 have potential to be useful for bioremediation of sites highly contaminated with petroleum hydrocarbons.The two genera of *Achromobacter* and *Ochrobactrum* are first to isolate and report in bioremediation petroleum hydrocarbon studies. More research is necessary tounderstand the fundamental mechanisms of enhancement and inhibition inthe microbial degradation of high concentration of toxic petroleum hydrocarbon compounds, these microorganisms could be used very effectively for *in situ*bioremediation in an environment which is highly contaminated with mineraloil. However, further research could be carried out on these strains, on genetic manipulation for improvement and exploitation as bioremediationvehicles.

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