Isolation and identification of bacteria biodegrading petroleum oil in various contaminated sites in Saudi Arabia

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ABSTRACT

This study was designed to isolate and purify a number of bacteria candidates from some sites contaminated with petroleum products, engine oil, lubrication oil, diesel oil and benzen in mechanic workshops and gas stations. Five mechanic workshops and five gas stations within Taif metropolitan King Saudi Arabia were selected and soil samples, were collected from each site. Samples were microbiologically analyzed using nutrient agar and enrichment media. Bacterial populations ranged between $2.1x10^2$ and $4.7\ x10^7$ CFU/g dry soil; being the highest in industrial area at Hawya. Total fungi ranged between $0.3x10^2$ and $5.9x10^3$ CFU/g dry soil with highest in Taif industrial area. Six isolates were taken, purified and named as GST1, GST8, MWST10, GST11, MWSH104 and MWSH203. Based on the morphological, physiological, biochemical characteristics, and APi kit the purified isolates were identified .The GST1 belonged to Erwinia species , GST8 and MWST10 to Bacillus species, GST11 to Pseudomonas species, MWSH104 to filamentous type of Actinomyces species and MWSH203 to Saccharomyces. The activity of the isolates GST8 (Bacillus subtilis) and GST11 (Pseudomonas putida) on 1 % (v/v) mineral oil was monitored and the growth rate, biomass yield, rate of degradation and physical appearances were used as indication for the ability of these isolates to grow on mineral oil. Both strains showed positive response up to 99.6%. The bacterial isolate GST11 was superior comparing to the isolate GST8. In addition, the genus-specific 16S-rDNA gene analysis was used to identify the isolates GST8 and GST11 at the molecular level. Using specific primers for 16S- rRNA gene of Bacillus and Pseudomonas, the expected fragments of 450 bp and 986 bp were amplified and sequenced. Alignment of deduced sequences using BLAST search showed that the closest match to the 450 bp fragment (88%) was with Bacillus spp 16S-rRNA (accession # HM046583-1). While, the best match to the 986 bp fragment (97%) was with Pseudomonas spp 16S-rRNA. (accession # EF451704).

Key words: *Engine oil, contaminated soil, pollution, Bacillus, Pseudomonas, 16S-rRNA.*

INTRODUCTION

Environmental pollution with petroleum and petroleum products (complex mixture of hydrocarbons) has been recognized as one of the most serious current problems especially when associated with accidental spills on large-scale. It is used to lubricate the parts of automobile engine in order to keep everything running smoothly (Hagwell *et al.*, 1992). Used oil was defined by the US Environmental Protection Agency (40CFR Pan 270) as oil that has been refined from crude oil or any synthetic oil; this has

been used and as a result of such use is contaminated by chemical impurities which contribute to chronic hazards including mutagenicity and carcinogenicity as well as environmental hazard with global ramifications (Blodgette, 2001).

Bioremediation has become an alternative way to remedy oil polluted sites, where the addition of specific microorganism (bacteria, cyanobacteria, algae, fungi and protozoa) or enhancement of microorganism already present, can improve biodegradation efficiency (Hagwell *et al.*, 1992). These microorganisms can degrade a wide range of target constituents present in oil sludge (Barathi and Vasudevan, 2001 and Mishra *et al.*, 2001). A large number of *Pseudomonas* strains capable of degrading polycyclic aromatic hydrocarbons have been isolated from soil (Kiyohara *et al.*, 1994 and Johnson *et al.*, 1996). Other petroleum hydrocarbon degraders include *Yokenella* spp., *Alcaligenes* spp., *Roseomanas* spp., *Sreanotrophomanas* spp., *Acinetobacter* spp., *Flavobacter* spp., *Cyanobacterium* spp., *Capnocytophage* spp., *Moraxella* spp. and *Bacillus* spp. (Antai, 1990). Other microorganisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent. However, they take longer time (Udeani *et al.*, 2009).

The vast range of substrates and metabolites present in hydrocarbon impacted soils surely provides an environment for the development of a quite complex microbial community (Butier and Mason, 1997). Microbial populations that consist of strains that belong to various genera have been detected in petroleum-contaminated soil or water (Sorkhoh *et al.*, 1995 and Chikere *et al.*, 2009). This strongly suggests that each strain and/or genus have their roles in the hydrocarbon transformation processes.

Microbial treatment can control contamination of soils with used or fresh petroleum products by reducing the length of the paraffin and oil molecules and by producing by-products that act as surfactants and paraffin and oil solvents. However, information on numbers and local species of microorganisms as well as their efficiency in degradation in Saudi Arabia is scarce.

In the present study, it is attempted first to survey total heterotrophic bacteria and fungi present in contaminated sites and to isolate and identify bacteria capable of degradation of used petroleum oil residuals. The capability of isolated bacteria to degrade mineral oil as a representative of hydrocarbon residuals was evaluated. In addition, morphological, physiological, biochemical characteristics tests and Api profile as well as the 16 S- ribosomal rRNA analysis were employed for the identification of the petroleum oil biodegrading-bacterial strains.

MATERIALS AND METHODS

Study site

Study sites were different gas stations and mechanic workshops in Taif metropoliton, KSA. These places are industrial areas in Taif city and Al Hawya city.

Sample collection and processing

Soil samples were collected from specific locations within the gas station or workshops that had heavy spillage of fresh or used engine oil, benzene, and diesel and lubrication oil. The locations had no growing grasses and soil samples were collected at three different locations at each workshop. One sample of 100g from each location was collected during fall 2009 and spring 2010. Samples were taken down to 10 cm depth, after discarding the upper 3 cm of the soil surface. Each soil sample was crushed, thoroughly mixed and sieved through a 2 mm sieve. Samples were placed in polyethylene

bags, closed tightly and stored at 4±1°C. The soils were characterized by hardened surfaces and blackish in color.

Bacterial and fungal enumeration

Total bacteria and fungi were enumerated by preparing ten fold serial dilutions of soil samples using physiological saline. One ml of each dilution was dropped onto Petri dishes then the nutrient agar and Sabbrauds media were poured. Plates were incubated for 48 – 72 h at 30°C. Numbers of viable microorganisms in the sample were determined.

Isolation and selection of degrading agents

The microorganisms used in this study were obtained by the enrichment culture technique from contaminated soils which had been contaminated with hydrocarbon for a long time at mechanic workshop and gas stations. Samples were obtained from different locations at Taif and Hawya. The medium used was Bushnell and Haas (1941) mineral medium. It contains (g/l) 0.2 $MgSO_4.7H_2O$; 1.0 K_2HPO_4; 1.0 KH_2PO_4; 0.05 $FeCl_3$; 1.0 NH_4NO_3; 0.02 $CaCl_2$; pH to 7-7.2 and sterilized at 15 psi for 15 min. Inoculation was done using Bushnell- Haas enrichment medium of 10 ml in 250 mL flasks into which ten grams of the contaminated soil was added and incubated at 30°C for 4 weeks. Samples that were turbid were sub-cultured into nutrient agar using LB broth, as diluents to observe the morphological characteristics of the isolates. Colonies showing a good growth and characters were picked and streaked on new Bushnell- Haas minimal agar plates. A rapidly growing, visually distinct colony and separate, morphologically unique isolates were selected for further analysis and purified by repeated plating.

Morphological, physiological and biochemical characterization of bacterial isolates

The morphological characteristics of the isolates were examined by Gram stain and biochemical reactions. The biochemical reactions include glucose fermentation, oxidase test, catalase production reaction, cell motility, egg yolk reaction and reaction in tryptose soya broth. Isolates were purified and named based on the morphological, physiological and the biochemical characteristics presented in Bergey's Manual of Determinative Bacteriology (Holt *et al.*,1994) and the APi Kit profiling, BioMerieux, France (Api, 2009).

Growth conditions

Medium used was Luria-Bertani (LB) broth (Trypton, 10g; yeast extract, 5g; NaCl, 5g; distilled water, 1000 ml) to grow mother cultures in 125 ml Erlenmeyer flasks with 20 ml of medium and incubated with shaking (150 rpm) at 37°C overnight. The isolate mother cultures were cultivated in a minimum salt medium containing 1 mL of mineral oil as a sole carbon and energy source. Cultures were cultivated in 250 ml Erlenmeyer flasks with 100 ml of medium and incubated with shaking (150 rpm) at 37°C.

Optical density and biomass measurement

The turbidity of 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 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 OD_{595} and dry mass was obtained.

Effect of mineral oil concentrations on bacterial growth activity

Growth of bacterial strains on different concentrations of mineral oil 1, 3, 5 % (v/v) was evaluated by the OD at 595 nm. Growth rate of cultures in exponential phase was 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 had the dimensions /h (Koch, 1984).

GC-FID methods and instrumentation

Samples were measured at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt. Samples were extracted by adding 100 μl of hexane and gentle mixing by continuous inversion for 10 min. The top phase containing the organic solvent with the extracted oil was removed with a Pasteur pipette and transferred to a new set of tubes, sealed and stored at -20°C until analysis. The analyses were carried out in triplicate in a Tech Comp D-7900 ver. 1.30 gas chromatograph equipped with a flame ionization detector using a 30 m x 0.32 mm, 0.25 μm internal diameter, polar DB1 fused silica capillary column (Supelco). The carrier gas was nitrogen at a flow rate of 20 ml/min. The temperature of the injector was 230°C and that of the flame ionization detector was maintained at 320°C. The oven temperature after sample injection (2μl) was 1 min at 50°C, increasing to 200°C at 15°C/min and held at this temperature for 2 min then raised to 280°C with an increasing rate of 15°C/min and held for 2 min.

Molecular identification of the bacterial isolates

To identify the isolates at the molecular level, the genus specific 16S- rRNA gene analysis using PCR was carried out. DNA was extracted from the over night culture of the isolates using the bacterial DNA preparation kit (Jean Bioscience, Germany). For the PCR reactions to amplify the 16S-rRNA gene of the isolates GST8 and GST11, two specific primers for the 16S-rRNA gene of *Bacillus* and *Pseudomonas* were selected. For *Bacillus*, the forward primer Com1 (5'CAGCAGCCGCGGTAATAC3') and the reverse primer Com2 (5'CCGTCAATTCCTTTGAGTTT3') were derived from data published by Lane *et al.* (1985). For *Pseudomonas*, the forward primers Ps-for (5'-GGTCTGAGAGGATGATCAGT-3') and reverse primer Ps-rev (5'-TTAGCTCCACCTCGCGGC-3']) were designed by (Alm *et al.*, 1996) according to the convention of the Oligonucleotide Probe Database (OPD). The PCR was performed in a 25μl reaction volume containing 1X PCR buffer (10mM Tris-HCl pH 8.3, 50mM KCl, 2mM MgCl2, 0.01% (w/v) gelatine), 250μM each of dGTP, dATP, dCTP and dTTP (dNTPs), 2.5 units of Taq DNA polymerases, 100 *p*mol of each primer and the DNA template. Thermocycling, which was conducted in an Eppendorf PCR system (Germany), started with an initial denaturation for 5 min at 94°C. A total of 35 cycles, each including 60 s at 94°C, 60 s at 50°C for *Bacillus* and 60 s at 52°C for *Pseudomonas*, and 90 s at 72°C, was followed by a final primer extension step of 5 min at 72°C. The purity and amount of PCR products were analyzed with 10 μl of the reaction mixture after agarose gel electrophoresis (1.5% agarose gel, including 0.5 μg of ethidium bromide ml^{-1}). Sequencing of the 450 and 1000 bp PCR product was done with the automated DNA sequencer (ABI, Gene line DNA sequencing, New York). The deduced sequence was aligned with the DNA data base available at the gene bank using the ncbi BLAST at (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

RESULTS AND DISCUSSION

Soil moisture ranged between 0.2 and 4.6 %, and pH between 2.3 and 7.6 (Table 1).

Total viable count

Total viable counts of bacteria and fungi were determined (Table 2). Total bacteria ranged between 2.1 $x10^2$ and 4.7 $x10^7$ CFU/g dry soil and the highest was in industrial area at Hawya. Total fungi ranged between $0.3x10^2$ and $5.9x10^3$ CFU/g being the highest in Taif industrial area. Ten soil samples were collected from 10 different mechanic workshops and gas stations. Results showed a prevalence rate of 10.0% yield of bacteria degrading agents.

Table (1): Moisture percentage and pH values of the contaminated soil.

Soil sample	Moisture%	pH
1	0.2	7.33
2	2.2	7.44
3	0.6	7.40
4	1.4	2.30
5	0.4	6.50
5	0.4	7.30
7	1.0	7.30
8	4.6	6.80
9	1.4	7.60
10	0.6	7.45

Table (2): Total viable counts of bacteria and Fungi in two industrial areas at Taif State, KSA.

Soil sample	Total bacterial counts	Total fungal counts
1	$2.9X\ 10^6$	$1.1X10^2$
2	$3.5X10^5$	$4.3X10^2$
3	$2.7X10^4$	$0.3X10^2$
4	$2.1X10^2$	ND
5	$8.8X10^6$	$5.9X10^3$
6	$4.7X1O^6$	$3.1X10^2$
7	$4.7X10^7$	$2.3X10^2$
8	$6.3X10^5$	$3.3X10^3$
9	$5.5X10^4$	$1.5X10^2$
10	$3.3X10^6$	ND

ND = not determined

Table (3): Morphological and biochemical characters of the obtained bacterial isolates.

Isolate	Color	Morphology	Gram reaction	Motility	Catalase	Oxidase	Proposed name
GST1	Clear	Short rod	-	+	+	-	*Erwinia spp.*
GST8	Creamy	Long rod Spore	+	+	+	-	*Bacillus spp.*
MWST10	Heavy Creamy	Long rod Spore	-	+	+	+	*Bacillus subtilis*
GST11	Clear greenish	Short rod	+	+	+	-	*Psuedomonas putida*
MWSH104	White	Long thin Hyphe	+	+	+	+	*Actinomyces*
MWSH203	Heavy Creamy	Oval	+	-	+	+	*Saccharomyces cerevieace*

Potential hydrocarbon degrader bacteria were isolated from soil samples that have been exposed to petroleum oil spills in mechanic workshops and gas stations. Saadoun (2002) reported that bacterial populations of polluted soils were between 9.5×10^5 and 237.5×10^5 CFU/g soil with 2 different colony types of bacterial strains which have been recovered on the agar plates. Results indicated that longer aged contamination exhibited a greater number of microorganisms. Udeani *et al.* (2009) evaluated the bacterial diversity of soil environment contaminated with used engine oil of mechanic workshops in Nigeria and found bacteria densities of 1.25×10^4 to 6.25×10^5 from the soil samples collected from each site.

Morphological, physiological and biochemical characterization of bacterial isolates

Phenotypic examination of bacterial isolates revealed that they belong mainly to the genera of *Bacillus, Psuedomonas, Actinomyces, Saccharomyces, Micrococcus*, *Erwinia* and *Enterobacter* (Fig. 1). Six isolates were identified on the basis of their cultural and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). The isolates were named as GST1, GST8, MWST10, GST11, MWSH104 and MWSH203. Based on their morpho-logical, biochemical characterization (Table 3), the isolates were identified as the following: GST1 as *Erwinia* species, GST8 and MWST10 as *Bacillus* species, GST11 as *Pseudomonas* species, MWSH104 to be filamentous type of *Actinomyces* species and MWSH203 as *Saccharomyces* species. Saadoun (2002) isolated *Pseudomonas*, *Enterobacter* and *Acinetobacter* from contaminated soils. Udeani *et al.* (2009) reported the isolation of *Bacillus Stearothermophilus* (8.3%) and *Cyanobacteria* (1.7%) from mechanic workshops at the sites sampled. Lazar *et al.* (1999) stated that microorganisms involved with the microbial treatment of crude oil, are generally live, naturally occurring, and are mainly facultative anaerobic, non-pathogenic, contain no sulphate-reducing bacteria or slime-forming bacteria and are environmentally safe. All of our bacterial strains, however, were catalase positive and aerobes except GST8 and MWST10 which were facultative anaerobics. In Addition, Lazar *et al.* (1999), Pokethitiyook *et al.* (2002) and Sadeghazad and Ghaemi (2003) reported that, *Pseudomonas* and *Bacilli* species were the most effective microorganisms in the biodegradation of heavy hydrocarbons. Accordingly, the two isolates GST8 and GST11 that were identified as *Bacilli* spp and *Pseudomonas* spp, respectively were selected to evaluate their ability to degrade the oil product residuals.

Growth rates

The specific growth rates of the isolates GST8 and GST11 on mineral oil (Figs. 2 and 3) showed that the former grew faster than the latter in mineral salts medium containing 1.0 % (v/v) mineral oil. The strain GST8 has a lag phase of approximately 6 h and peak growth at 10 h reaching 0.001 g/ml (optical density of 1 at 595 nm) (Fig. 2). Maximum specific growth rate (μ_{max}) for the strain GST8 was 0.243 h^{-1} (Table 4).

Table (4): Growth rates of the selected strains grown in minimal medium with mineral oil as carbon and energy source.

Strains	Growth rate (μ) (h^{-1})	Biomass yield (g cells /mL mineral oil)
GST 8	0.065	2.74
GST 11	0.911	2.96

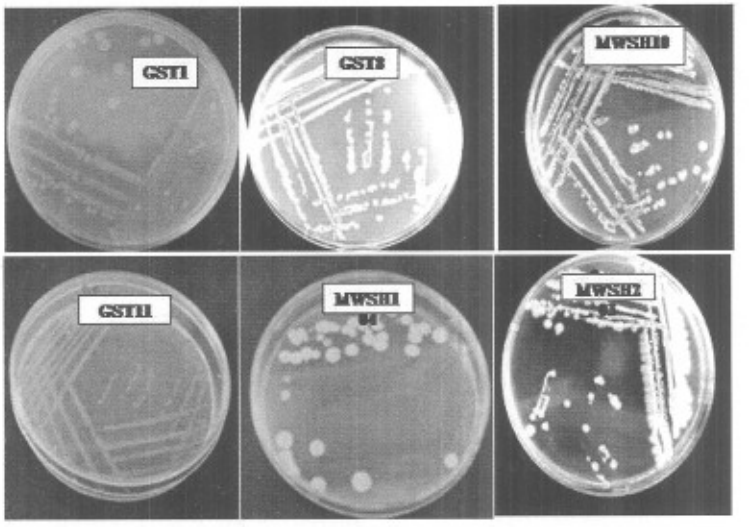

Fig. (1): Subcultures of pure bacterial isolates from soils contaminated with used oil products.

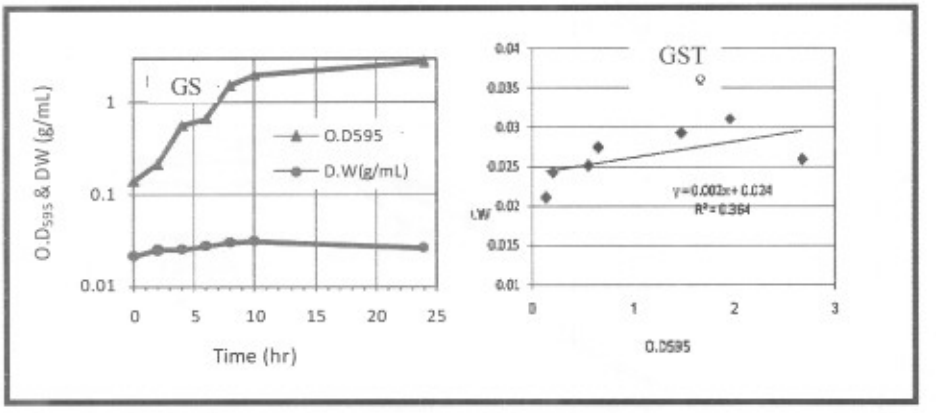

Fig. (2): Growth curve and relation between biomass and optical density of isolate GST8.

Biomass and optical density

Optical density and biomass yield of bacterial strains were determined simultaneously. The linear relation between OD_{595} and biomass was obtained during growth on 1% of mineral oil as shown in Figs. (2 and 3). These figures indicate the specific growth rate and biomass precipitation on a period of bacterial cultivation in mineral salts medium containing 1 % (v/v) mineral oil.

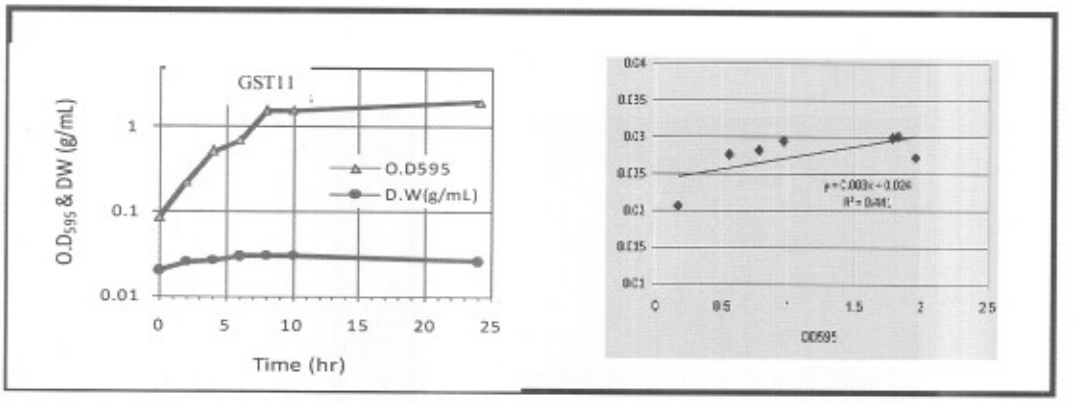

Fig. (3): Growth curve and relation between biomass and optical density of strain GST11.

Individual microorganisms can metabolize only a limited range of hydrocarbon substrates, so, assemblages of mixed populations with overall broad enzymatic capacities are required maximize to the rate and extent of petroleum biodegradation maximise (Ghazali *et al.* 2004). Microbial populations consist of strains belonging to various genera have been detected in petroleum-contaminated soil or water (Sorkhoh *et al.*, 1995). This strongly suggests that such strains have their roles in the hydrocarbon transformation processes. The vast range of substrates and metabolites present in hydrocarbon impacted soils surely provides an environment for the development of a quite complex microbial community (Butier and Mason, 1997). It is used to lubricate the parts of automobile engine in order to keep everything running smoothly (Hagwell *et al.*, 1992).

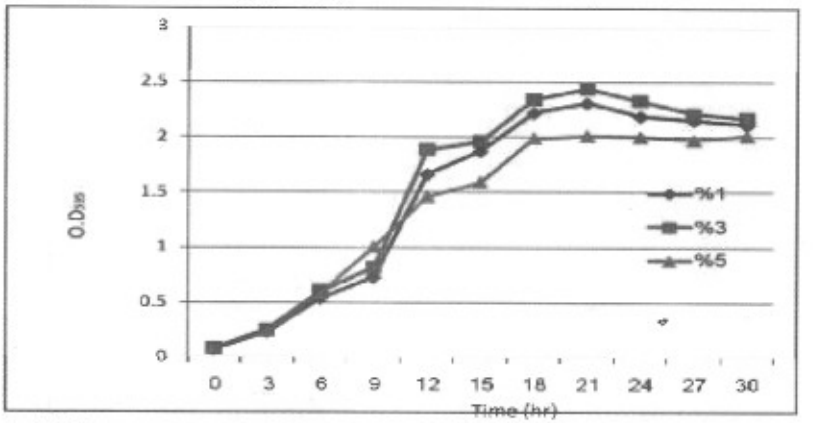

Fig. (4): Effect of different concentrations of mineral oil on growth of strain GST11.

The effect of substrate concentration

In order to determine the optimum hydrocarbon concentration for microbial growth, further growth rate experiments at different concentrations of mineral oil, 1, 3 and 5% (v/v) were carried out. Growth of bacterial strains was evaluated by measuring culture optical density (OD) at 595 nm. The strains showed different growth rates in different media (data not shown). Fig. (4) shows the concentrations and maximum growth rate values of strain GST11. Most growth occurred in the initial 20h. The concentration of 3% was the optimum and had a μ_{max} of 0.963 h^{-}.

Etoumi (2007) observed a reduction in wax appearance temperature and heavy hydrocarbon fractions by biodegradation of paraffinic hydrocarbons using *Pseudomonas* and *Actinomyces* species. It was reported that the lower the concentration of hydrocarbons the higher was the utilization.

Degradation of mineral oil by the bacterial strains

The standard cultures of *Bacillus subtilis* and *Pseudomonas putida* strains were examined for their capability to degrade mineral oil as sole carbon source and energy in mineral medium for 24 h. Both exhibited high efficiency of assimilating the mineral oil. Strain GST11 showed the highest degradation rate (95.65 %) after 10 h but strain GST8 reached the highest rate after 24 h (96.45 %) (Fig.5). Mechanisms by which bacteria are able to degrade oil products were described by Lazar *et al.* (1999). First, bacteria can metabolise the existing paraffin oil, and then partially digest the paraffin by breaking the chemical bonds between carbon atoms in the chain until the paraffin becomes more liquidized once again. Once this is achieved, the bacteria start to work on other paraffin molecules as such they do not actually consume the oil.

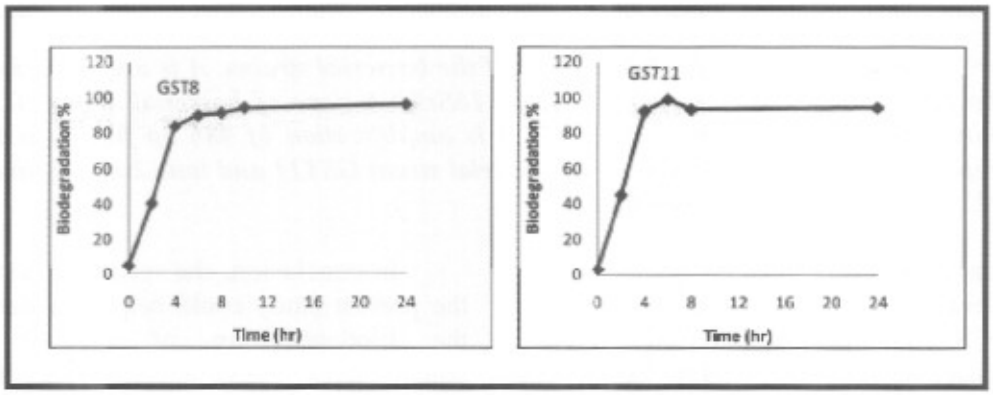

Fig. (5): GC analysis of 1% (v/v) concentration of mineral oil treated with the two strains GST8 and GST11.

Second, biosurfactants which are produced by the bacteria are another contributing factor to the breakdown of paraffins. Finally, bacteria are extremely motile and their movement in oil fluids enhances their effectiveness in the break up of paraffins. Isolation of alkane degrading microorganisms from oil contaminated soil has been reported by several researchers. Nazina *et al.* (2005) have obtained hydrocarbon oxidizing *Geobacilli* strains from formation waters of oil fields. Hydrocarbon degrading members of the family *Bacillaceae* were found to dominate the oil contaminated soil of

Kuwait (Mohamed *et al.*, 2006). Moreover, the thermophilic bacterial strain *G. thermodenitrificans* that shows selective degradation of long chain alkanes, similar to the degradation pattern of isolate GST11, was also isolated (Wang *et al.*, 2006).

Molecular identification of bacterial isolates GST8 and GST11

A fragment of the appropriate size (450 bp) was amplified from the genomic DNA of the isolate SGT8 by PCR using the specific primers for *Bacillus* genera (com1 and com2) (Fig 6-A). For the isolate SGT11, a fragment of 986 bp was amplified by PCR using the specific primers for *Pseudomonas* as shown in Figure (6-B). The size of amplified product is identical to the expected amplified fragment 450 bp and 986 bp, for *Bacillus* and *Pseudomonas* spp, respectively.

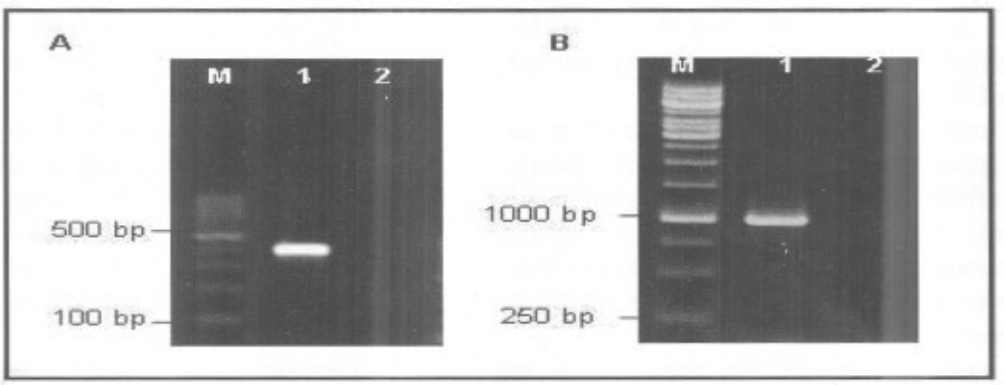

Fig. (6): PCR amplification of 16SrRNA genes of the bacterial strains. A is amplification of 450 bp fragment (Lane 1) representing the 16SrRNA gene of bacterial isolate GST8 and lane 2 is negative control (E. coli.). B is amplification of 986 bp fragment (Lane 1) representing the 16SrRNA gene of bacterial strain GST11 and lane 2 is negative control (E. coli.) M is DNA size marker.

Sequence analysis

Nucleotide sequence for the 450 bp and 986 bp PCR products were determined (Fig. 7). The Blast search for the 450 bp DNA segment showed closest match (88%) with *Bacillus* spp 16S-rRNA (accession # HM046583-1). The Blast search for the 986 bp DNA segment showed closest match (97%) with *Pseudomonas spp* 16S-rRNA (accession # EF451704). These results (at the molecular level) confirmed the identification (at the morphological and biochemical level) of the bacterial strains as *Bacillus subtlis* and *Pseudomonas putida.*

In conclusion, the results obtained from the present study could help in understanding the biodegradation of mineral oil in contaminated sites, as well as to design efficient biocatalyst allowing transformation of oil fractions into valuable compounds. The isolation of pure strains from such a consortium has also been achieved; their mineral oil degradation ability was confirmed, and different effects of mineral oil on their degrading capacity have been shown. Preliminary identification of isolated strains has been achieved and further work continues on their molecular characterization.

More research is required to understand the fundamental mechanisms of enhancement and inhibition of the microbial degradation of high concentration of toxic compounds. However, these microorganisms could be used very effectively for *in situ* bioremediation in an environment which is highly contaminated with oil products. However, further research could be carried out on genetic manipulation of bacterial isolates for improvement and exploitation as bioremediation vehicles.

Fig. (7): The deduced DNA sequence of the 16SrRNA gene of the bacterial isolates GST 8 and GST 11. A is DNA sequence of the 450 bp fragment representing the 16SrRNA gene of the bacterial isolate GST8 and B is DNA sequence of the 986 bp fragment representing the 16SrRNA gene of the bacterial isolate GST11.

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الملخص العربي

عزل وتوصيف البكتريا المحللة لزيوت البترول فى بعض المناطق الملوثة بالمملكة العربية السعودية

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تم تنفيذ هذه الدراسة لعزل وتنقية بعض العزلات البكتيرية من الأماكن الملوثة بمنتجات البترول (زيت الموتور، زيت التشحيم ، زيت الديزل ، البنزين) فى محطات البنزين وورش ميكانيكا اصلاح السيارات. حيث تم اختيار 5 ورش ميكانيكا اصلاح سيارات و5 محطات بنزين فى مدينة الطائف بالمملكة العربية السعودية. تم تجمع و تحليل العينات باستخدام بيئات الأجار المغذى وبيئات الأستكثار. وتم العد باستخدام تخفيفات عشرية من10^{-7}- 10^{-1} لكل عينات التربة الملوثة. ووجد ان المحتوى البكتيرى يتراوح مابين $2.1x10^{2}$ - 4.7×10^{7} CFU/g تربة جافة. وكانت اعلى الأعداد فى منطقة الحوية. وكانت اعداد الفطريات تتراوح مابين $0.3x10^{2}$ - $5.9x10^{3}$ CFU/g تربة جافة و كانت اعلى الأعداد فى المنطقة الصناعية بالطائف. وتم عزل وتنقية 6 عزلات سميت GST1, GST8, MWST10, GST11, MWSH104, MWSH203 وباستخدام اختبارات الخواص المورفولوجية والفسيولوجية وطريقة *API kit* وجد ان هذه العزلات تابعة لأجناس *Erwinia, Bacillus, Pseudomonas, Actinomyces, Saccharomyces* . تم دراسة تأثير السلالات GST8, GST11 التابعة لجنسى *Bacillus, Pseudomonas* على الزيت المعدنى 1% واستخدم معدل النمو والوزن الجاف ومعدل التكسير الحيوى كدليل على قدرة العزلات على النمو فى الزيت المعدنى. واظهرت كلا السلالتين استجابة موجبة K_c 99.6% تكسيرا حيويا ، وكانت السلالة GST11 سباقة مقارنة بالسلالة GST8. واستخدم تحليل 16S-rDNA لتأكيد تعريف السلالتين GST8, GST11 على المستوى الجزيئى. وباستخدام بادئات primers خاصة لجين 16S- rRNA لجنسى *Bacillus, Pseudomonas* تم تكبير قطع الدنا المتوقعة ذات الوزن الجزيئ 450 و 986 زوج من القواعد. و بعد تحديد تتابع القواعد النيتروجينية لقطعتى الدنا وبمقارنتها بالتتابعات الموجودة فى بنك الجينات باستخدام بحث BLAST, أظهر البحث ان اقرب نظير الى الشظية 450 bp كان الجنس *Bacillus* بنسبة 88% (رقم الكود HM046583-1) وكان أقرب نظير للشظية 986 bp هو الجنس *Pseudomonas* بنسبة 97 % (رقم الكود EF451704).