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Analysis for *Streptomyces* spp. recovered from oil refinery soils to grow on diesel

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ABSTRACT

Aims: The investigation aimed to examine the crude oil-contaminated soil *Streptomyces* flora and study their capability to grow on diesel fuel as a sole carbon source and their analysis for the presence of alkane hydroxylase gene (*alkB*) by PCR.
Methodology and results: A total of 17 *Streptomyces* isolates were recovered from hydrocarbon-contaminated soil samples on starch casein nitrate agar medium with the ability of 4 isolates to grow on diesel [0.1 % (v/v)] as assessed by agar plate diffusion method, enzymatic assay and dry weight measurements. The ability of the four isolates (JR2b, JR3a, JR5b, and JR6f) to grow on diesel was revealed by the colour change of the reaction mixture and showing a growth response by growing around diesel-containing wells with a percentage increase in the dry weight of 24.60, 26.23, 18.03, and 18.03 after 28 days of incubation as compared to zero time, respectively. Although the four isolates were capable to degrade diesel as indicated by the three assessment techniques, they did not show any PCR product.
Conclusion, significance and impact of study: The isolates that grew on diesel and showed no PCR product might not contain the *alkB* gene, which implies that *alkB* gene is not the only gene that is responsible for the degradation of alkanes.

Keywords: Degradation, diesel, soil, *Streptomyces*

INTRODUCTION

Biodegradation of petroleum hydrocarbons by soil microorganisms has been an area of interest for many years (Kok and Oldenhuis, 1989; Mukherji *et al.*, 2004). Isolation and identification of microorganisms responsible for hydrocarbon biodegradation have long been recognized and a number of hydrocarbon-degrading bacteria, yeasts, fungi, and algae are therefore available (Beilen *et al.*, 2003; Jonathan *et al.*, 2003). Some reports indicated that *Streptomyces* flora could play a very important role in degradation of hydrocarbons (Radwan *et al.*, 1998; Barabas *et al.*, 2001). For instance, Aitken *et al*. (1998) isolated *Streptomyces* spp. from polycyclic aromatic hydrocarbons contaminated soil that have the ability to degrade phenanthrene. Later, Barabas and his colleagues (2001) have isolated 3 *Streptomyces* strains (*S. griseoflavus*, *S. parvus*, and *S plicatus*) from the Kuwait Burgan oil field with the ability to utilize *n*-hexadecane, *n*-octadecane, kerosene, and crude oil as sole carbon and energy sources.

Bacterial degradation of alkane usually occurs through the sequential oxidation of one or both terminal methyl groups of the molecule, first to an alcohol, then to an aldehyde, and finally to a fatty acid (Marín *et al.*, 2001). A simple and rapid method was developed by Saadoun (2002; 2004) and Saadoun *et al*. (2008a) to assess the potential of organisms to grow on alkanes by detection of alcohol-production as a result of such degradation.

Molecular assessment of the ability of *Streptomyces* to degrade hydrocarbons usually done by PCR through targeting presumably involved genes. Many targeted genes have been studied to assess the ability of bacteria to degrade hydrocarbon compounds. One of the most studied genes is *alkB* gene which codes for a membrane-bound monooxygenase or alkane hydroxylase that plays the first step in alkane degradation process (Belhaj *et al.*, 2002). However, more attention was devoted to alkane hydroxylase genes as markers to predict the potential of different organisms for oil degradation (Maeng *et al.*, 1996; Murrell *et al.*, 2000; Hamamura *et al.*, 2001; Maier *et al.*, 2001; Padda *et al.*, 2001; Pandey *et al.*, 2001; Iida *et al.*, 2002; Sluis *et al.*, 2002; Smits *et al.*, 2002). Genes involved in alkane degradation from bacteria *Acinetobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Rhodococcus*, and from yeasts (*Candida* and *Yarrowia*) were cloned and sequenced (Watkinson and Morgan, 1990).

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In Jordan few studies have been conducted on hydrocarbon-polluted environments. Saadoun (2002; 2004) have isolated different bacteria from soil contaminated with oil and showed their ability to degrade diesel and short chain alkanes. Later, Saadoun *et al*. (2008a) have isolated *Streptomyces* spp. from Safawi/Jordan soils contaminated with crude oil. This study aimed to examine the crude oil-contaminated soil *Streptomyces* flora and study their capability to grow on diesel fuel as a sole carbon source via enzymatic analysis and examine their content of the alkane hydroxylase gene (*alkB*) by PCR.

MATERIALS AND METHODS

Location, sampling and sample processing

This study describes the occurrence and recovery of streptomycetes in chronically oil-polluted soils of Jordan Refinery in the City of Zarqa/Jordan with a historic exposure to crude or fuel oil spills for more than 25 years.

Samples from Jordan Refinery were collected from four different sites contaminated with crude/fuel oil spills. A control sample was taken from the refinery area but away from all sites. After removing approximately 3 cm of the soil surface material, ~ 220-950 g of soil were collected and placed in plastic bags and stored in the laboratory at 4 ±1 °C until use.

Processing and characterization of the sample

Collected soil samples were crushed thoroughly mixed and sieved through a 2 mm pore size mesh (Retsch, Haan, Germany) to get rid of large debris. The sieved soil was used for determination of color, pH, total petroleum hydrocarbons, and soil moisture in addition to the isolation of *Streptomyces*.

Soil pH, moisture and total petroleum hydrocarbon (TPH) measurements

Soil suspension was made by diluting the soil to a known volume of distilled water (1:2 w/v) and the pH was measured using a regular pH meter at 25 °C (Hanna, Italy). For moisture determination, one gram of each soil sample was dried at 65 °C in the oven (WTB Binder, Germany) and the difference in the weight of the sample before and after drying was considered as the moisture content. Total petroleum hydrocarbon (TPH) in these contaminated soil samples was determined by constitutive extraction of 10 g of soil with hexane, dichloromethane and chloroform (100 mL each). All of the three extracted portions were pooled and dried at room temperature by evaporation of solvent in a fume hood (CMS, Spain). After solvent evaporation the amount of residual TPH was determined gravimetrically (Williams *et al.*, 1972).

Treatment of soil samples and isolation technique

Enumeration and isolation of *Streptomyces* spp. were performed as described by Saadoun *et al*. (2008b) to represent those that were able to grow on starch casein nitrate agar (SCNA) plates.

Assay for diesel degradation

Growth conditions of Streptomyces and their adaptation on diesel

Each *Streptomyces* isolate that was recovered from the contaminated soils was inoculated into 50 mL of adaptation broth (0.1% yeast extract, 0.1% peptone and 0.1% (v/v) diesel), then incubated at 28 °C with shaking at 100 rev/min for 24 h. The whole mixture in each flask was centrifuged for 5 min at 4000 rev/min and the pellet was suspended in the same medium and incubated under the same conditions for 24 h. The last step was repeated three times, followed by washing the cells three times with 0.1 mol/L phosphate buffer, pH 7.5. The pellets were finally suspended in a small volume (5 mL) of phosphate buffer and stored until used.

Growth on diesel

Growth of diesel-adapted *Streptomyces* was determined by the hole-plate diffusion method as previously described by Saadoun (2002) and Saadoun *et al*. (2008a) and evaluated at 7-day intervals by measuring the dry weight of cells/mL of the cell suspension. Briefly, 20 μL of the diesel-adapted *Streptomyces* were inoculated into 50 mL mineral salts medium (MSM) (Leadbetter and Foster, 1958) supplemented with 0.05% (v/v) filter-sterilized diesel and incubated in water bath shaker (100 rev/min) for 28 days at 28 °C. Dry weight was performed after placing 20 mL of the final bacterial cell suspension in pre-weighed aluminum tares in the oven for 12 h at 65 °C before weighing.

Enzymatic assay for diesel degradation

Biodegradation of diesel was assayed according to the method of Jacobs *et al*. (1983) and as described by Saadoun (2002) and Saadoun *et al*. (2008a). Change in the color was compared with four controls. The first control contained no diesel (substrate), the second contained no NAD^+ and the third contained no cells. A fourth control consisted of heating the cells for 10 min at 90 °C. The reaction was followed for 24 h and examined after 1, 2, 6, 12 and 24 h.

PCR study

Growth conditions

All *Streptomyces* isolates that showed diesel degradation potential were cultured on Oxoid tryptic soy broth (TSB)

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(Hopowood *et al.*, 1985) (30 g/L) at 28 °C with shaking at 140 rev/min for 48 h. Purity of the cultures was confirmed by plating 0.1 mL from the broth on starch casein nitrate agar (SCNA) (Küster Williams, 1964) plates and incubated at 28 °C for 72 h.

Extraction of genomic DNA from pure Streptomyces isolates

Genomic DNA extraction was conducted using Wizard Genomic DNA Purification Kit (Promega, USA). Approximately 40 mg (wet weight) mycelia were used in case of *Streptomyces* isolates, whereas one mL of an overnight broth growth was used in case of other bacteria. All DNA manipulation, handling and PCR work was conducted using DNase, RNase-Free barrier tips (Promega, USA).

Quantitation of the extracted DNA and estimation of its purity

The isolated DNA was checked for purity and quantitated spectrophotometrically (Sambrook *et al.*, 1989). In each extraction group one sample (selected randomly) was extracted twice and was used in the estimation process. One sample (50 μL) was diluted in 950 μL Tris EDTA (TE) buffer and measured at 260 and 280 nm wavelengths (Genesys 2, Milton Roy, USA). The readings at 260 nm were used to calculate the DNA concentration in the original sample, where 1 OD corresponds to 50 μg/mL of double-stranded DNA. The ratio between the readings at 260 nm and 280 nm (OD_{260} /OD_{280}) was used to estimate the purity of the DNA.

Design of primer sets for detection of alkane hydroxlyase gene (alkB)

The alkane hydroxylase gene (*alkB*) sequence of seven bacterial strains were obtained from GenBank and subjected to multiple alignments. Highly homologous regions were selected to design PCR primer set (Kohno *et al.*, 2002). Two primers (Operon Technologies, USA) were used in the PCR study and as described before by Saadoun *et al*. (2008a). These primers were ALK-F: 5´-TCGAGCACAACCGCGGCCACCA-3´ and ALK-R: 5´-CCGTAGTGCTCGACGTAGTT-3´.

PCR amplification

Amplification reactions were performed in volumes of 25 μL containing 12.5 μL of master mix (Promega, USA), 1.5 μL forward primer, 1.5 μL reverse primer, 8.5 μL nuclease free water and 1 μL of template DNA. PCR amplification was carried out in 0.2 mL thin walled, nucleases free water PCR tubes (Treff lab, Switzerland) using iCycler theromocycler (Bio-Rad, USA) programmed as followed: initial denaturizing step at 95 °C for 2 min followed by 30 cycles with denaturation at 94 °C for 60 sec, annealing at 40 °C for 30 sec, and extension at 72 °C for 30 sec, final extension at 40 °C for 3 min. After that, the tubes were held at 4 °C for direct use or stored at -20 °C for later use.

Electrophoresis and photography

Electrophoresis was carried out in 2% agarose (Promega, USA) gels in 1× TBE buffer at 100 V for 1.5 h. The size of the PCR products was estimated using 100 bp DNA ladder (Promega, USA) and detected on the agarose gel by staining with ethidium bromide (EB) (Acros Organic, USA) at 0.5 μL/mL final concentration. Gels were viewed and documented using Gel Doc (Bio-Rad, USA).

Characterization of *Streptomyces* isolates

Streptomyces isolates with the positive potential to grow on diesel were characterized morphologically and physiologically according to the International *Streptomyces* project (ISP) (Shirling and Gottlieb, 1966) and as described by as described by Saadoun *et al*. (2008b).

RESULTS

Characterization of soil samples

The collected samples were characterized in terms of color, pH and moisture. The color of the samples ranged from light brown to black while pH ranged from 6.04–6.92 and moisture content ranged from 2.4 to 6.0% (Table 1). Total petroleum hydrocarbons (TPH) were measured for all the samples and were found between 75 and 142 mg/g of soil (Table 1). As a control, unpolluted soil sample was evaluated for color, TPH and moisture. The samples showed a light brown color, contained 45 mg/g TPH and a relatively lower moisture content of 6.78%.

Occurrence, count and color diversity of *Streptomyces*

Streptomyces population in the polluted soils varied between 2×10^4 and 5×10^4 C.F.U./g (Table 1). However, this count in the unpolluted soils was 2×10^4 C.F.U./g (Table 1). Control soil sample which was taken from apparently non-polluted area showed slightly similar counts with an average of 2×10^4 C.F.U./g dry soil (Table 1).

Assay for diesel degradation

Physical appearance

Results demonstrated the ability of four *Streptomyces* isolates to grow on diesel when compared to the control well in the same plate that contains sterile distilled water only (Figure 1).

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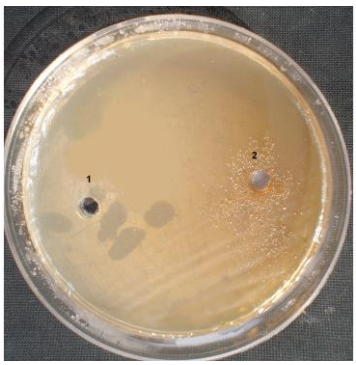


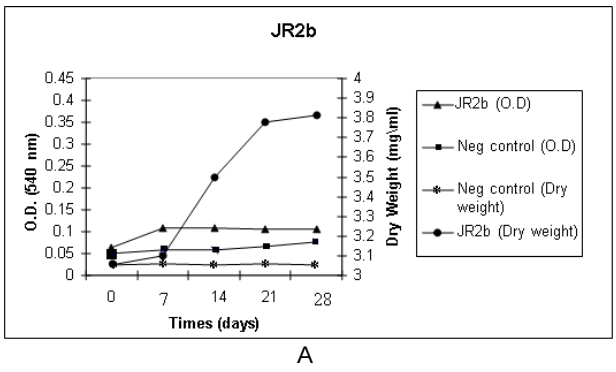
Figure 1: Growth response of *Streptomyces* isolates on diesel as indicated by physical appearance/ growth response of isolate No. JR6f in well 2, well 1 contained sterile distilled water.

Growth on diesel

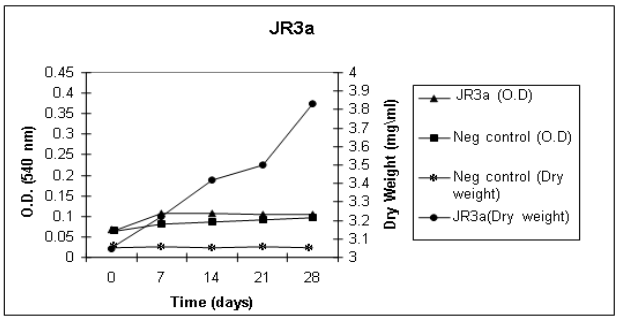
Growth on diesel was evaluated by measuring the dry weight of *Steptomyces* after the incubation period. Figure 2 shows the growth response of JR2b, JR3a, JR5b, and JR6f *Streptomyces* isolates on diesel fuel as indicated by the increase in the dry weight with a percentage increase of 24.60, 26.23, 18.03, and 18.03 after 28 days of incubation as compared to zero time, respectively. However, by using this technique, we were unable to determine which isolate had the most ability to grow on diesel. Nevertheless, by estimating the dry weight of the cells we were able to determine which isolate have the most growth ability during the different intervals of incubation. It is worth noticing that degradation of diesel does not appear to be linear during all the time intervals.

Enzymatic assay

Table 2 shows that four of the tested active isolates were able to degrade diesel as indicated by color change from blue to yellow. All of the tested isolates were able to change the color of 2, 6-dichlorophenolindophnol (DCPIP) from blue color to yellow after 6 h of incubation. The four isolates performed similarly in degradation with no differences observed between them as indicated by the color change from blue to yellow-green in the first 2 h of incubation and then to yellow after 6 h. The ability of Streptomyces to degrade diesel enzymatically was tested by either the removal of NAD+ from the reaction mixture (control 2) or heating the cells for 10 min at 90 °C (control 4) with the subsequent inability of the isolate to perform degradation as indicated by the no change in the final color of the reaction mixture. Results of control 2 and control 4 experiments clearly show that the color of the reaction mixtures for all tested isolates did not change to yellow and they remain green at 6, 12, and 24 h.



A



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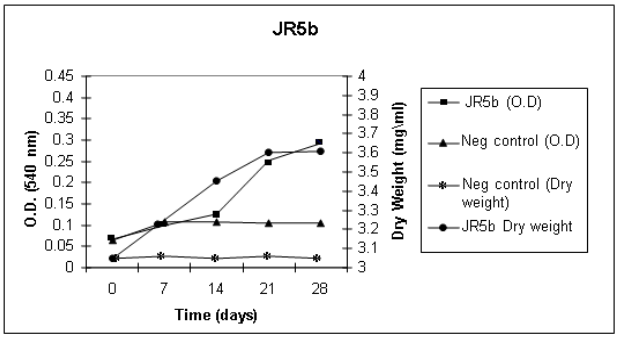
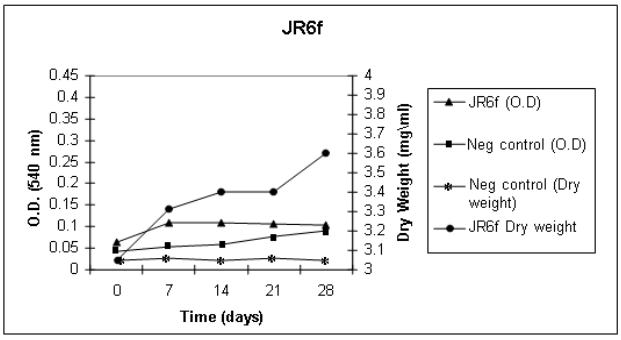


C



D

Figure 2: Dry weight (mg/mL) measurements and O.D. readings at 540 nm for the isolates JR2b (a); JR3a (b); JR5b (c); JR6f (d).

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Detection of *alkB* gene sequence in *Streptomyces* isolates by PCR

Genomic DNA was extracted using commercial DNA isolation kit. The extracted DNA was of a good quality as being tested by agarose gel electrophoresis. The extracted DNA is intact, clean and free of RNA (data not shown).

PCR study for catabolic *alkB* gene

Figure 3 shows the PCR products for four isolates (JR2b, JR3a, JR5b, and JR6f) which grew around diesel-containing wells. All isolates (JR2b, JR3a, JR5b, and JR6f) did not show any PCR product although they were able to grow on diesel.

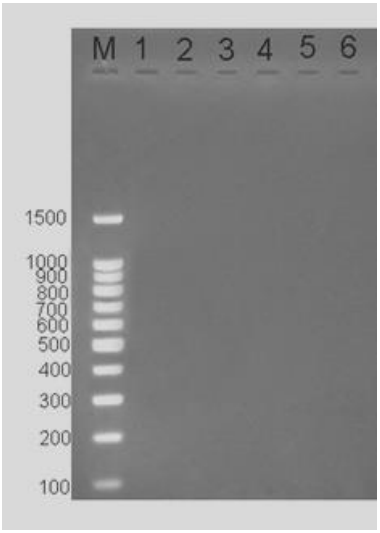

Figure 3: Agarose gel (2%) electrophoresis of PCR amplification of alkane hydroxylase genes from *Streptomyces* isolates with Alk primer. Lane M: molecular weight marker 100 bp, lane 1: (negative control no PCR products), lane 2: negative control (no DNA template), lane 3: JR2b, lane 4: JR3a, lane 5: JR5b, lane 6: JR6f.

Isolation and characterization of *Streptomyces* isolates

A total of 17 different *Streptomyces*-like colonies were recovered from the polluted soils (Table 1). Results of dilution series and plate count of the samples that harbored the *Streptomyces* isolates revealed an average *Streptomyces* count ranged between 2×10^4 and 5×10^4 colony forming unit (CFU) /g dry soil (Table 1). The colony morphology of the *Streptomyces* isolates on starch casein nitrate agar plates (SCNA) after 10 days of incubation at 27 °C indicated that they were small (1-10 mm diameter), discrete and leathery, initially relatively with smooth surface but later developed a weft of aerial mycelium that appeared granular, powdery and velvety (data not shown).

Cultural and morphological characterization of these isolates resulted in their distribution into two color series (Table 1). The isolates JR3a and JR2b showed the maximum growth on diesel after 28 days with a dry weight biomass of 3.80 and 3.85 mg/mL, respectively (Figure 2A and Figure 2B). However, both JR5b and JR6f isolates showed the minimum growth on diesel after 28 days with a dry weight biomass of 3.60 mg/mL (Figure 2C and Figure 2D). The isolates JR2b, JR3a, JR5b, and JR6f showed a considerable growth on diesel with the ability of JR5b and JR6f isolates to utilize all of the tested sugars except rhamnose.

Those isolates that were able to utilize diesel were further characterized (Table 3). These isolates are with either spiral, or flexuous, or biverticillus sporophore and were unable to produce soluble and melanin pigments. Two isolates (JR2b, JR3a, and JR5b) showed distinctive reverse side color. Sugar utilization test indicate that all of the isolates were able to utilize glucose, sucrose, and fructose Isolates JR5b and JR6f utilized all of the tested sugars except rhamnose.

DISCUSSION

The work presented here has successfully isolated five potential soil *Streptomyces* isolates from oil-contaminated soil and assessed for their ability to degrade diesel via measuring their growth response, enzymatic assay, in addition to PCR analysis of the alkane hydroxylase gene (*alkB*) that is usually linked to hydrocarbon degradation.

Streptomyces are widely known for their capabilities to degrade organic compounds and have shown a capability to degrade hydrocarbon compounds (Radwan *et al.*, 1998; Barabas *et al.*, 2001; Saadoun *et al.*, 2008b). These degradation capabilities are important metabolic activities that deserve to be studied particularly if these organisms are able to grow in polluted soils.

All the pH and moisture content values are considered within the optimal range for microbial activities. The variation in the color, pH or the moisture could be due to differences in levels and types of organic matter as well as differences in precipitation rates and exposure to sun. On the other hand, the difference in TPH could be due to lower or no exposure to oil spills.

Hydrocarbons spilled on soil elevate levels of organic carbon and may either serve as substrates for microbial growth or prove toxic to microbial growth and activity (Bossert and Barth, 1984). In our study, low streptomycetes counts in soils from Jordan Refinery suggest the failure of these indigenous microorganisms to grow and adapt due to accumulation of TPH which could reach toxic levels due to the nature of the crude oil or fuel-oil and the plethora of toxic substances it contained**.** Control soil sample which was taken from apparently non-polluted area showed slightly similar counts with an average of 2×10^4 C.F.U./g dry soil. As mentioned earlier, this variation could be due to higher concentration of pollutant and location of sampling. In addition, the occurrences of *Streptomyces* in different habitats are affected by many factors including pH, temperatures,

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nutrients and moisture (El-Nakeeb and Lechevalier, 1963).

All of *Streptomyces* isolates were screened for their potential to grow on diesel fuel by the hole-plate diffusion method as described by Saadoun (2002) and Saadoun *et al.* (2008b) using mineral salt medium supplemented with diesel fuel (0.1% v/v) as carbon source. This method allowed direct determination of the ability of the recovered *Streptomyces* isolates to grow on diesel.

Table 1: Hydrocarbon-contaminated soil samples collected from Jordan Oil Refinery and their characters and *Streptomyces* content.

Sample	Soil sample characters					Bacterial count			*Streptomyces* color diversity	
No.	Colour	Weight (kg)	Moisture %	pH	TPH mg/g	Total count CFU X10^6/ g soil	*Streptomyces* count CFU X10^4/g soil	% of *Streptomyces*/gm soil	White	Grey
JR 1	Black	0.765	2.4	6.04	117	0.34	2	5.88	1	1
JR 2	Black	0.700	3.8	6.72	99	0.22	5	22.73	5	
JR 3	Black	0.227	6.0	6.65	142	0.35	3	8.57	3	
JR:6	Light brown	0.221	3.6	6.92	75	0.64	5	7.81		5
JR 5 (control)	Light brown	0.309	2.2	6.78	45	0.79	2	2.53		2

Table 2: Action of different *Streptomyces* isolates on diesel fuel as indicated by color change[a]

		Streptomyces isolate			
Reaction condition	Time (h)	JR2b	JR3a	JR5b	JR6f
+NAD$^+$ 29 °C + Diesel	1	Y-Gr	Y-Gr	Y-Gr	Y-Gr
	2	Y-Gr	Y-Gr	Y-Gr	Y-Gr
	6	Y	Y	Y	Y
	12	Y	Y	Y	Y
	24	Y	Y	Y	Y
Result		+	+	+	+
-NAD$^+$ 29 °C (Control 2)[b]	1	Bl	Bl	Bl	Bl
	2	Bl	Bl	Bl	Bl
	6	Gr	Gr	Gr	Gr
	12	Gr	Gr	Gr	Gr
	24	Gr	Gr	Gr	Gr
Result		-	-	-	-
+NAD$^+$ 90 °C (Control 4)	1	Bl	Bl	Bl	Bl
	2	Bl	Bl	Bl	Bl
	6	Gr	Gr	Gr	Gr
	12	Gr	Gr	Gr	Gr
	24	Gr	Gr	Gr	Gr
Result		-	-	-	-

[a]Color change from dark blue to other colors at different time interval by each *Streptomyces* isolate: Y=yellow; Gr=green; Bl=blue.

[b]The first control contained no diesel (substrate), the third contained no cells. Control 2: NAD$^+$ is removed from the reaction mixture, and control 4 cells were heated for 10 min at 90 °C.

To further evaluate the ability of the isolates to degrade diesel, *Streptomyces* growth and utilization of diesel was enzymatically assayed following the procedure of Jacobs *et al.* (1983). A pre-adapting step for *Streptomyces* isolates to utilize diesel fuel was carried out before testing diesel oxidation was intended for the induction of mixed function oxygenases and other catabolic enzymes essential for biodegradation (Saadoun, 2002). The ability of *Streptomyces* to degrade diesel enzymatically was tested by either the removal of NAD$^+$ from the reaction mixture or heating the cells for 10 min at 90 °C with the subsequent inability of the isolate to perform degradation as indicated by the no change in the final color of the reaction mixture. These results appeared to be in accordance with previous results reporting the same enzymatic test on *Pseudomonas* strains (Saadoun, 2004) and *Streptomyces* strains (Saadoun *et al.*, 2008b).

To test for the presence of *alkB* gene in the isolated *Streptomyces* a pair of primers were designed by multiple alignments of several sequences from all alkane hydroxylase genes registered in the Gene Bank. This allows the detection of the maximum number of alkane degrading bacteria. Alkane hydroxylase gene (*alkB*) is a very important gene that has been recorded in several actinomycetes linked to hydrocarbon degradation including *Nocardia*, *Mycobacteria* and *Rhodococcus* genera (El-Nakeeb and Lechevalier, 1963; Hopowood *et al.*, 1985; Kohno *et al.*, 2002), and *Streptomyces* spp. (Saadoun *et al.*, 2008b). There were 16 alkane hydroxylase gene sequences in the data bank, and all were classified into 3 groups through phylogenetic analysis (Saul *et al.*, 2005). Currently over 250 *alkB* gene homologues were found in diverse bacterial species in which a large portion of these genes was detected in oil-contaminated environments (Padda *et al.*, 2001).

The five tested isolates that did not show any PCR product but were able to grow on diesel may be explained that the *alkB* gene is not the only gene that is responsible for the degradation of alkanes, and the isolates that grew on diesel and showed no PCR product might not contain the *alkB* gene or the product might be merely a none specific annealing and extension to the primers rather than specific amplification. On the other hand, it could be a similar but nonfunctional gene, thus, the isolates were not showing PCR band. The presence of alkB gene in *Streptomyces* isolates does not mean that they can degrade diesel as diesel contains many hydrocarbon components other than alkanes.

The results of the high occurrence of the white and grey series is in agreement with other studies by Saadoun

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Table 3: Characteristics of the *Streptomyces* isolates that grew on diesel.

Isolate No.	Macroscopic cultural characteristics					Utilization of carbon sources[d]					
						I	Ra	X	A	M	Rh
	AM[a]	SM[b]	Soluble pigment	Melanin pigment	Sporophore morphology[c]						
JR2b	Beige	+	-	-	F	-	+	-	+	-	+
JR3a	Beige	+	-	-	F	+	+	+	-	+	-
JR5b	Grey	+	-	-	BIV	+	-	+	+	+	+
JR6f	Grey	-	-	-	S	+	-	+	+	+	+

[a]AM: Aerial mycelium
[b]SM: Substrate mycelium; -: non-distinctive; +: distinctive
[c]Sporophore morphology: Flexuous (F), Biverticillus (BIV), and Spirales (S),)
[d]All of the tested isolates were able to utilize glucose, sucrose, and frcuctose. I=Insitol, Ra=Raffinose, X=Xylose, A=Arabinos, M=Mannose, and Rh=Rhamnose.

and Al-Momani (1997) and Saadon et al. (1999) which found that the grey and white color were the most frequent color series in Jordanian soils.

CONCLUSION

The study showed that *Streptomyces* flora does prevail in oil-contaminated soil with a potential to degrade diesel as assessed by different physical, chemical and molecular techniques. The presence of *alkB* gene in *Streptomyces* isolates does not mean that they can degrade diesel.

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CONFLICT OF INTEREST

The authors of this manuscript have declared no conflict of interest.

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