Environ Sci Pollut Res (2012) 19:812–820
DOI 10.1007/s11356-011-0624-z

RESEARCH ARTICLE

Indigenous soil bacteria with the combined potential for hydrocarbon consumption and heavy metal resistance

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Received: 19 May 2011 / Accepted: 15 September 2011 / Published online: 25 September 2011

Abstract
Introduction Transconjugant bacteria with combined potential for hydrocarbon utilization and heavy metal resistance were suggested by earlier investigators for bioremediation of soils co-contaminated with hydrocarbons and heavy metals. The purpose of this study was to offer evidence that such microorganisms are already part of the indigenous soil microflora.
Methods Microorganisms in pristine and oily soils were counted on nutrient agar and a mineral medium with oil as a sole carbon source, in the absence and presence of either sodium arsenate (As V), sodium arsenite (As III) or cadmium sulfate, and characterized via 16S rRNA gene sequencing. The hydrocarbon-consumption potential of individual strains in the presence and absence of heavy metal salts was measured.
Results Pristine and oil-contaminated soil samples harbored indigenous bacteria with the combined potential for hydrocarbon utilization and As and Cd resistance in numbers up to 4×10^5 CFU g^{-1}. Unicellular bacteria were affiliated to the following species arranged in decreasing order of predominance: *Bacillus subtilis*, *Corynebacterium pseudotuberculosis*, *Brevibacterium linens*, *Alcaligenes faecalis*, *Enterobacter aerogenes*, and *Chromobacterium orangum*. Filamentous forms were affiliated to *Nocardia corallina*, *Streptomyces flavovirens*, *Micromonospora chalcea*, and *Nocardia paraffinea*. All these isolates could grow on a wide range of pure aliphatic and aromatic hydrocarbons, as sole sources of carbon and energy, and could consume oil and pure hydrocarbons in batch cultures. Low As concentrations, and to a lesser extent Cd concentrations, enhanced the hydrocarbon-consumption potential by the individual isolates.
Conclusion There is no need for molecularly designing microorganisms with the combined potential for hydrocarbon utilization and heavy metal resistance, because they are already a part of the indigenous soil microflora.

Keywords Arsenic · Bacteria · Cadmium · Oil utilization · Pollution

1 Background, aim, and scope

Before their withdrawal from Kuwait in February 1991, the Iraqi forces blew up more than 700 oil wells in the Kuwaiti desert. Crude oil remained gushing through about 7 months forming about 300 “oil lakes” covering an area of about 50 km^2 (McKinnon and Vine 1991). Immediately after the liberation, oil was pumped out of many such lakes and exported, but the lake beds remained for about two decades now polluted with sediments to depths reaching about 60 cm. It is known that oil- (Barringer et al. 2005, 2006; Wiatrowski et al. 2006) and pesticide- (Grassi and Netti 2000; Protano et al. 2000) polluted areas are usually simultaneously polluted with heavy metals. In this context, many pesticides are hydrocarbon derivatives. There are numerous literature reports on hydrocarbon-utilizing soil bacteria, e.g., species belonging to the genera *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Chromobacterium*, *Enterobacter*, *Micromonospora*, *Nocardia*, *Streptomyces* and many others (for a recent review, see Radwan 2009). Similarly, there are many reports on heavy

Responsible editor: Zhihong Xu

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metal resistant and detoxifying bacteria, e.g., *Acinetobacter*, *Bacillus*, *Mycobacterium*, *Pseudomonas*, *Rhododcoccus* and many others (Nies 1999; Barkay et al. 2003; Chiu et al. 2007). Much fewer reports, mainly from our laboratory, described bacteria, e.g. *Alcanivorax*, *Bacillus*, *Gordonia*, *Dietzia*, *Pseudomonas* and others (Sorkhoh et al. 2010a, b), and haloarchaea, e.g. *Halobacterium*, *Halococcus*, and *Haloferax* (Al-Mailem et al. 2010a, b) with the combined potential for hydrocarbon utilization and mercury resistance and volatilization.

Earlier workers suggested the construction of trans-conjugant bacteria with the combined potential for hydrocarbon utilization and heavy metal resistance (Yoon 2003), or isolating them from the environment (Yoon 1998; Dua et al. 2002), and using them for bioremediation purposes. It is however, well known that inoculating microorganisms into an already established ecosystem (bioaugmentation) is not an easy job. Therefore, the reliance upon indigenous microorganisms with such combined potential, if feasible, could be the proper option for bioremediation. In view of the occurrence of crude oil in the Arabian Gulf region since geological ages, it may be assumed that the Gulf desert areas may harbor such microflora. The major objectives of this paper were to test the validity of this assumption and to study the effects of the two heavy metals, arsenic and cadmium on the hydrocarbon utilization and consumption potential of the predominant microorganisms. These two elements were studied on the basis of their frequent association with oil pollution in nature (Barringer et al. 2005, 2006; Wiatrowski et al. 2006). The results would be interesting for basic science and useful in constructing and enhancing bioremediation approaches for the contaminated desert areas.

2 Materials and methods

2.1 Sampling

Three visually oil-contaminated desert soil samples about 30 m apart were taken, 5 cm below the surfaces, from the Khazma area, north of Kuwait. For comparison, another three visually pristine (sediment free) samples were similarly taken from a nearby unpolluted area. Samples were collected in sterile polyethylene bags, transported in an ice box (about 8°C) to the laboratory and processed the same day. The As and Cd contents of the soil samples were determined quantitatively (Takeuchi et al. 2005). The samples were first digested with conc. HNO_3 and H_2SO_4 on hot plates, and the heavy metals were determined using inductively coupled plasma mass spectrophotometer (ICP-MS, Varian-820-MS, NJ, USA) using the reference soil standard IV-ICP-MS-71D.

2.2 Enumeration and characterization of microorganisms

Cultivable bacteria with the combined potential for oil-utilization and arsenic and cadmium resistance were counted adopting the standard dilution-plating method. For this, we used a specific mineral medium (Sorkhoh et al. 1990) with oil vapor as sole source of carbon and energy and various concentrations of sodium arsenate (As V); 0–300 mM, sodium arsenite (As III); 0–30 mM or cadmium sulfate; 0–10 mM. The mineral medium had the following composition (g l^{-1}): 1.0 $NaNO_3$, 0.6 KH_2PO_4, 0.9 Na_2HPO_4, 0.2 K_2SO_4, 0.4 $MgSO_4{\cdot}7H_2O$, 0.7 $CaCl_2{\cdot}2H_2O$, 2.5 ml of trace elements mixture (g l^{-1}): 2.3 $ZnSO_4$, 1.8 $MnSO_4$, 0.6 H_3BO_3, 1.0 $CuSO_4$, 0.4 Na_2 MoO_4, 0.4 $CoCl_2$, 0.7 KI, 1.0 EDTA, 0.4 $FeSO_4$, 0.004 $NiCl_2$. To solidify the medium 1.5% agar was added and the medium pH was adjusted to 7 with HCl. A 1 g portion of the studied soil sample was aseptically vortexed for 5 min in 99 ml sterile water to have the stock concentration of 10^{-1}. A series of dilutions (10^{-3}, 10^{-4} and 10^{-5}) was prepared using sterile water. Aliquots of 0.1 ml from the different dilutions were spread on the solid mineral medium without and with different concentrations of sodium arsenate, sodium arsenite or cadmium sulfate. For each dilution, three replicate plates were inoculated. Oil vapor was created by impregnating filter papers in the plate covers with 2 ml aliquots of Kuwaiti light crude oil and sealing the plates immediately after inoculation. Although oil vapor comprises only low molecular weight hydrocarbons ($<C_9$ compounds; Al-Mailem et al. 2010a, b), the oxygenases (also called hydroxylases) involved in the initial step of microbial attack on those compounds are known to attack low, medium, and high molecular weight hydrocarbons (Rehm and Reiff 1981). Incubation was at 30°C for 14 days. Microorganisms with conventional carbon source requirements were counted on conventional nutrient agar (Mast Group Ltd, Merseyside, UK) following a similar procedure. Total colony forming units (CFU's) in plates containing between about 50 and 250 colonies were counted. The mean values of the three replicate plates were calculated and, taking the dilution factor into consideration, the numbers per gram soil were obtained. It was confirmed that the colonies which grew on the oil-medium failed to grow on the same medium in the absence of oil vapor, and that they did not bring about any "pitting" in the agar medium, i.e. they were not agarolytic (Sorkhoh et al. 2010a, b). The three replicate plates were pooled and similar microbial strains in these plates were recognized by their colony morphologies, microscopic characteristics and staining reactions. They were counted and their proportions of the total CFU's calculated. Since the total colony numbers always exceeded 600 colonies, it was not practical to make molecular analysis (sequencing) for all of them.

Therefore, five colonies representative of each strain were isolated, purified and used for the sequencing study.

Representative strains were studied for their 16S rDNA sequences. Total genomic DNA was extracted from 48 h biomass using the GenElute bacterial genomic DNA kit (Sigma, USA) following the manufacturer's protocol. The 16S rRNA genes were PCR-amplified. The 550-bp fragment was amplified with the universal combined primers 907R (5′-CCCCGTCAATTCMTTTGAGTTT-3′) and GM5F (5′-CCTACGGGAG GCAGCAG-3′; Teske et al. 1996). PCR was conducted using the GenAmp PCR Core Reagents kit (Applied Biosystem, USA). The PCR products were purified using the QIA purification kit (Qiagen, USA) and sequenced using the BigDye Terminator V.1 cycle sequencing kit and the ABI 3130xl genetic analyzer (Applied Biosystem, USA). These partial 16S rRNA gene sequences are available under the GenBank accession numbers listed in Table 2. The sequences were compared with their nearest GenBank matches.

2.3 Hydrocarbon utilization and consumption potential

To determine the range of pure hydrocarbons which individual representative microorganisms could utilize, the isolates were tested for growth on the solid mineral medium, containing 0.1% of individual pure alkanes with chain lengths: C_9, C_{12}, C_{16}, C_{20}, C_{24}, C_{32}, and C_{40} and the aromatic hydrocarbons phenanthrene, naphthalene, and biphenyl, as sole sources of carbon and energy. Individual pure hydrocarbons were first dissolved in diethylether and the calculated solution volumes were spread on solid medium surfaces. The solvent was subsequently volatilized aseptically by nitrogen flush. A common inoculum was prepared for each tested strain by homogenizing one loopful of 48-h-old biomass in 5 ml sterile water. One loopful of the inoculum was streaked on the pure hydrocarbon containing medium. After 10 days of incubation at 30°C, the cultures were examined for growth.

The hydrocarbon-consumption potential of the representative strains was quantitatively determined by gas–liquid chromatography (GLC). For this purpose, crude oil, *n*-octadecane as representative of aliphatics and phenantherene as a representative of aromatics were used. Liquid mineral medium, 200 ml portions, were dispensed in 500-ml conical flasks, and provided with 20 mg portions of the tested hydrocarbon. Flasks were inoculated with 0.2 ml aliquots of the common inoculum and sealed. The flasks were incubated on an electric rotary shaker at 120 rpm and room temperature, about 25°C for 2 weeks. Three replicates were prepared throughout. The residual hydrocarbons were recovered from the media with three successive portions of 5 ml pentane. The combined extracts were completed to 20 ml with pentane and 1 μl was analyzed by GLC. Hydrocarbon consumption was expressed in terms of percentage total peak area reduction based on the total peak areas of the controls (similarly prepared, but using autoclaved inocula). The GLC was done using a Chrompack (NJ, USA) CP-9000 instrument equipped with FID, a WCOT fused silica CP-Sil-5CB capillary column, 15 m× 0.25 mm and a temperature program of 45–310°C, raising the temperature 10°C min^{-1}, using nitrogen as carrier gas. The detector temperature was 250°C and the injector temperature 300°C. This method was also used to determine hydrocarbon consumption in media containing different concentrations of sodium arsenate, sodium arsenite and cadmium sulfate, and to obtain the hydrocarbon contents of soil samples using *n*-hexadecane as an external standard.

3 Results

As expected, the quantitative analysis showed that the oily soil, with 5.8 mg kg^{-1} of As and 4.1 mg kg^{-1} of Cd was more heavily polluted with the two heavy metals than the pristine samples with only 0.8 mg kg^{-1} and 0.1 mg kg^{-1} of As and Cd, respectively. The total hydrocarbon contents of the oily and pristine soil samples were 208.6 mg g^{-1} and 11.3 μg g^{-1}, respectively; they consisted predominantly of *n*-alkanes longer than C_{19}.

The results in Table 1 show that both the pristine (oil-free) as well as the oil-polluted soil samples contained sodium-arsenate-, sodium-arsenite-, and cadmium-sulfate-resistant bacteria. We differentiated in this table the smooth, shiny, slimy colonies of unicellular bacteria on one hand and the rough, powdery, slightly medium-dipped colonies of filamentous bacteria on the other hand. As expected, the total numbers of CFU's per gram of both colony types were higher on the "collective", nutrient agar medium than on the specific, mineral medium with oil vapor as a sole carbon source. Obviously, the microorganisms growing on the latter medium containing sodium arsenate, sodium arsenite or cadmium sulfate were those with the combined potential for hydrocarbon utilization and heavy metal resistance. A common result was that the numbers of CFU's decreased with increasing heavy-metal salt concentrations in the media, and that the colonies on the heavy-metal-free media were considerably larger in size than their counterparts on the heavy-metal-containing media.

Table 2 presents the identities of the representative isolates, their frequencies and the highest heavy metal salt concentrations they tolerated, in addition to data related to the 16S rDNA sequences of the those microorganisms. The bacterial species listed in the table, which belong predominantly to the subdivision actinobacteria occurred in the same orders of frequency both in pristine and oil-polluted soils. The highest sodium arsenate, sodium arsenite and

Table 1 Total numbers of As- and Cd-resistant, oil-utilizing -bacteria in oil-free and oil-polluted soils

	Number of colony forming units $\times 10^3$ g^{-1} soil			
	On nutrient agar		On oil-containing media	
	Unicellular bacteria	Filamentous bacteria	Unicellular bacteria	Filamentous bacteria
Sodium arsenate added to the medium				
Oil-free soil (mM)				
0	402±21	313±18	31±1.1	35±1.3
50	368±15	301±17	23±1.0	20±0.9
100	277±11	212±8	10±0.4	7±0.2
300	192±7	115±6	8±0.3	3±0.1
Oily soil (mM)				
0	399±12	315±16	6±0.4	22±1.0
50	231±11	241±12	4±0.1	17±0.6
100	211±10	197±9	3±0.1	10±0.5
300	176±7	152±6	1±0.1	4±0.1
Sodium arsenite added to the medium				
Oil-free soil (mM)				
0	398±15	311±14	32±1.0	31±1.2
5	217±10	189±9	25±1.1	22±0.9
10	138±7	112±6	18±0.8	19±0.8
20	79±3	62±2	7±0.3	6±0.2
Oily soil (mM)				
0	388±18	321±16	21±1.0	15±0.6
5	217±10	248±12	18±0.8	8±0.4
10	131±7	113±6	10±0.5	6±0.2
20	97±4	79±5	5±0.2	4±0.1
Cadmium sulfate added to the medium				
Oil-free soil (mM)				
0	410±14	220±8	13±0.6	10±0.4
0.01	300±15	190±9	12±0.7	9±0.5
0.1	120±6	110±5	8±0.4	5±0.2
1.0	10±1	20±1	4±0.1	2±0.0
Oily soil (mM)				
0	448±21	284±13	16±0.8	12±0.5
0.01	440±22	280±14	16±0.9	12±0.6
0.1	220±10	140±6	10±0.5	8±0.3
1.0	30±1	40±1	0	1±0.0

Values are means of triplicates each; ±standard deviation

cadmium sulfate concentrations tolerated by the individual species were on oil-containing media less than on conventional nutrient agar.

The qualitative growth tests of all strains separately on the individual pure hydrocarbons, showed that the following individual species grew on the solid mineral medium containing all individual aliphatic (*n*-alkanes: C_9 through C_{40}) and aromatic (phenanthrene, biphenyl, naphthalene) hydrocarbons as sole sources of carbon: *Bacillus subtilis*, *Corynebacterium pseudotuberculosis*, *Enterobacter aerogenes*, *Nocardia corallina*, and *Streptomyces flavovirens*. *Brevibacterium linens* and *Micromonospora chalcea*, grew on all *n*-alkanes, but failed to grow in any of the aromatics as sole substrates. *Alcaligenes faecalis* and *Nocardia paraffinea* failed to grow on *n*-alkanes longer than C_{21}, and *Chromobacterium orangum* failed to grow on phenanthrene and naphthalene, but grew successfully on biphenyl in addition to all *n*-alkanes as sole substrates.

The quantitative determinations by GLC (Table 3) showed that all isolates could consume considerable proportions of crude oil, *n*-octadecane (as a representative of aliphatics) and phenanthrene (as a representative of aromatics) in nutrient broth provided with a mixture of 10 mM sodium arsenate and 0.3 mM cadmium sulfate.

Table 2 16S rDNA sequencing of As-resistant and Cd-resistant soil bacteria

Strains code numbers	% of the total CFU	Highest heavy metal salt concentration (mM) tolerated		Nearest GenBank match (subdivision)	Bases compared (bp)	% Similarity	GenBank accession
		On nutrient agar	On oil-containing medium				
Sodium arsenate							
Unicellular bacteria							
AVO1	32	80	60	*Bacillus subtilis* (F)	537	99	JF710618
AVO2	24	400	160	*Corynebacterium pseudotuberculosis* (A)	538	100	JF710619
AVO3	19	400	80	*Brevibacterium linens* (A)	524	100	JF806515
AVO4	15	80	60	*Alcaligenes faecalis* (β)	527	99	JF710620
AVO5	6	40	40	*Enterobacter aerogenes* (γ)	496	98	JF710621
AVO6	4	400	80	*Chromobacterium orangum* (β)	514	99	JF710622
Filamentous bacteria							
AVO7	38	400	100	*Nocardia corallina* (A)	510	100	JF710623
AVO8	28	320	80	*Streptomyces flavovirens* (A)	500	99	JF806513
AVO9	20	80	40	*Micromonospora chalcea* (A)	492	99	JF710624
AVO10	14	160	40	*Nocardia paraffinea* (A)	470	99	JF806514
Sodium arsenite							
Unicellular bacteria							
AIIIO1	30	30	10	*Bacillus subtilis* (F)	537	99	JF710618
AIIIO2	26	5	10	*Corynebacterium pseudotuberculosis* (A)	538	100	JF710619
AIIIO3	16	20	5	*Brevibacterium linens* (A)	524	100	JF806515
AIIIO4	16	10	5	*Alcaligenes faecalis* (β)	527	99	JF710620
AIIIO5	7	30	5	*Enterobacter aerogenes* (γ)	496	98	JF710621
AIIIO6	5	30	5	*Chromobacterium orangum (*β)	514	99	JF710622
Filamentous bacteria							
AIIIO7	40	30	10	*Nocardia corallina* (A)	510	100	JF710623
AIIIO8	26	30	10	*Streptomyces flavovirens* (A)	500	99	JF806513
AIIIO9	22	30	5	*Micromonospora chalcea* (A)	492	99	JF710624
AIIIO10	12	30	5	*Nocardia paraffinea* (A)	470	99	JF806514
Cadmium sulfate							
Unicellular bacteria							
COB1	32	1.8	0.9	*Bacillus subtilis* (F)	540	99	JF710618
COB2	24	1.8	0.6	*Corynebacterium pseudotuberculosis* (A)	510	98	JF710619
COB3	18	1.8	0.9	*Brevibacterium linens* (A)	545	100	JF806515
COB4	12	1.5	0.3	*Alcaligenes faecalis* (β)	490	100	JF710620
COB5	8	1.5	0.6	*Enterobacter aerogenes* (γ)	506	99	JF710621
COB6	6	1.8	0.9	*Chromobacterium orangum* (β)	515	99	JF710622
Filamentous bacteria							
COA7	41	1.5	0.9	*Nocardia corallina* (A)	510	100	JF710623
COA8	29	1.5	0.6	*Streptomyces flavovirens* (A)	500	99	JF806513
COA9	19	1.5	0.9	*Micromonospora chalcea* (A)	492	98	JF710624
COA10	11	1.5	0.6	*Nocardia paraffinea* (A)	470	99	JF806514

AV sodium arsenate, *AIII* sodium arsenite, *C* cadmium, *O* oil utilizes, *F* Firmicutes, *A* Actinobacteria, *β* Betaproteobacteria, *γ* Gammaproteobacteria

Table 3 Consumption of crude oil and pure hydrocarbons by As- and Cd-resistant bacteria

Isolate identities	% Consumption in nutrient broth containing 10 mM sodium arsenate and 0.3 mM cadmium sulfate		
	Crude oil	*n*-Octadecane	Phenanthrene
Unicellular bacteria			
Bacillus subtilis	39.5±1.7	47.6±2.3	36.2±1.6
Corynebacterium pseudotuberculosis	35.2±1.6	32.6±1.5	37.1±1.6
Brevibacterium linens	39.4±1.9	45.1±2.2	31.3±1.4
Alcaligenes faecalis	30.6±1.3	29.9±1.4	32.2±1.5
Enterobacter aerogenes	31.5±1.4	45.3±2.0	28.9±1.4
Chromobacterium orangum	29.4±1.4	35.2±1.7	24.2±1.1
Filamentous bacteria			
Nocardia corallina	43.2±2.1	35.2±1.6	44.3±2.1
Streptomyces flavovirens	39.1±1.8	43.3±2.0	37.2±1.7
Micromonospora chalcea	30.2±1.4	34.3±1.5	23.3±1.1
Nocardia paraffinea	32.4±1.5	45.2±2.1	28.2±1.3

Values are means of triplicates each; ±standard deviation. Incubation was for 2 weeks

Figure 1 shows the effects of sodium arsenate and cadmium sulfate concentrations on the crude oil consumption rates by the individual representative isolates. Sodium arsenate at concentrations of up to 20 mM enhanced the oil consumption by all strains; sodium arsenite did that with four strains. Higher arsenate and arsenite concentrations in the medium gradually inhibited the microbial oil consumption. The oil consumption by *B. subtilis* (♦) and, albeit to a less extent by *N. corallina* (◊) was somewhat enhanced, or remained rather constant. Regarding the consumption by *C. pseudotuberculosis* (■), any increase in cadmium sulfate concentration gradually inhibited the hydrocarbon consumption. Oil consumption rates by *S. flavovirens* (□), *M. chalcea* (Δ) and *N. paraffinea* (○) were rather similar over a wide range of cadmium sulfate concentrations (0–0.9 mM). *A. faecalis* (−), *C. orangum* (●), and *B. linens* (▲) showed highest oil consumption rates in the absence of cadmium sulfate, whose increasing concentrations gradually inhibited oil consumption by the three bacteria. The microorganism with the highest hydrocarbon-consumption rates in the presence of the highest heavy metal concentrations (40–80 mM sodium arsenate and 0.6–1.2 mM cadmium sulfate) was *N. corallina* (Δ). In general, the filamentous bacteria seemed to be more effective in oil consumption than the unicellular bacteria in the presence of the highest cadmium sulfate concentration tested, namely 1.2 mM.

4 Discussion

According to Kulkarni and Deshmukh (2001), heavy metals occupy globally a leading position as environmental pollutants next to pesticides. Because they are highly toxic to a wide range of biological systems, heavy metals are expected to be inhibitors of microorganisms involved in self-cleaning and bioremediation of xenobiotic-contaminated areas. Therefore, some studies were concerned with isolating bacterial strains (Yoon 1998; Dua et al. 2002) or constructing new "designer" transconjugant bacteria with the combined potential for heavy metal resistance and biodegradation of xenobiotics (Yoon 2003). However, as mentioned above, in view of the well-known difficulty of introducing "designer" strains into an already established and equilibrated microbial "ecosystem", emphasis should practically be put on indigenous rather than "introduced" strains. The former have the advantage of being already adapted to the "established" environmental conditions and equilibria, and thus, have the chance to be the best survivors.

With all these facts in mind, a relevant question arises regarding the natural occurrence, frequencies and identities of microorganisms with the combined potential for heavy metal resistance and hydrocarbon utilization, both in pristine soils and soils with long history oil pollution.

It is interesting that oil-polluted Kuwaiti desert samples and nearby pristine samples were rather rich in sodium-arsenate-, sodium-arsenite- and cadmium-sulfate-resistant bacteria. Confirming literature reports (Jackson et al. 2005), several strains could resist extreme concentrations of sodium arsenate, up to 400 mM, but only in the absence of oil. Also consolidating the latter report, arsenite was more toxic to the bacterial isolates than was arsenate. The fact that the total numbers of As-resistant bacteria on the conventional nutrient agar medium were in the magnitude of 10^5 CFU g^{-1}, but on the mineral medium with oil vapor as a sole carbon source in the magnitude of "only" 10^3–10^4 CFU g^{-1} implies that cultivable microorganisms with the combined potential for As and Cd resistance and oil

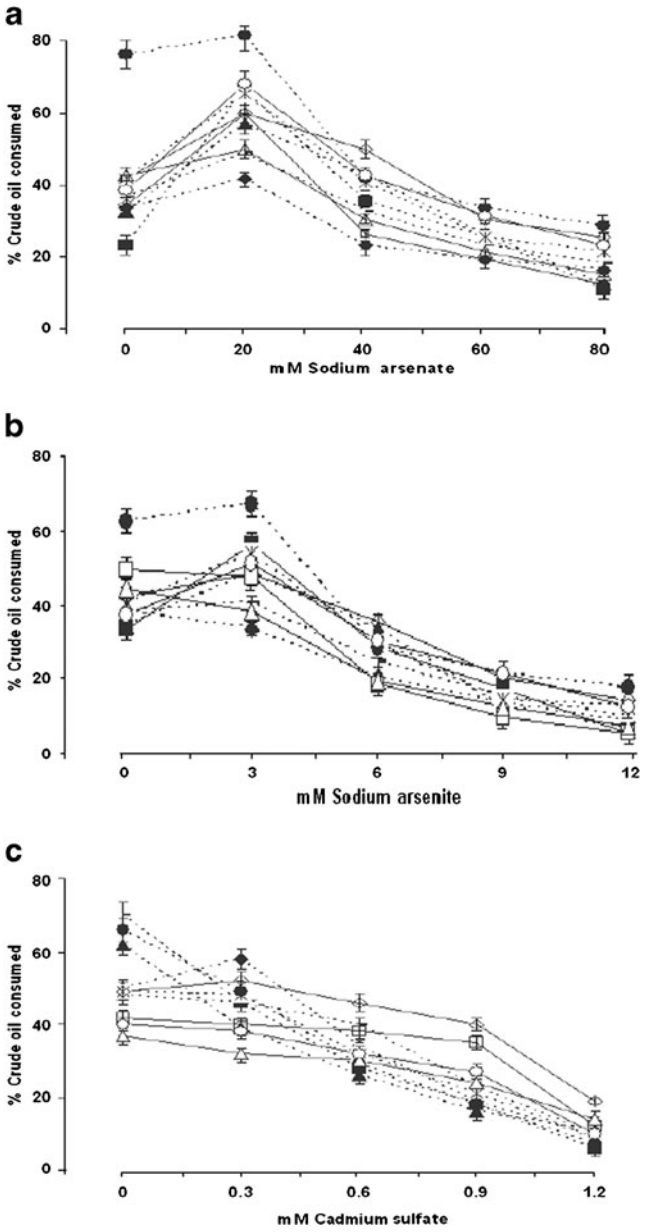

Fig. 1 Effects of sodium arsenate, sodium arsenite, and cadmium sulfate concentrations on oil consumption by individual bacteria and actinomycetes. Bacteria, *broken lines*; actinomycetes, *solid lines*. **a** Sodium arsenate; **b** Sodium arsenite; **c** Cadmium sulfate. *Bacillus subtilis*, *closed diamonds* (♦); *Corynebacterium pseudotuberculosis*, *closed squares* (■); *Brevibacterium linens*, *closed triangle* (▲); *Alcaligenes faecalis*, *open minus* (−); *Enterobacter aerogenes*, *open stars* (*), *Chromobacterium orangum*, *closed circles* (●); *Nocardia corallina*, *open diamonds* (◊); *Streptomyces flavovirens*,*open squares* (□); *Micromonospora chalcea*, *open triangles* (Δ); *Nocardia paraffinea, open circles* (○)

utilization (which grew on the mineral medium + oil vapor) make up between 1% and 10% of the heavy-metal-resistant strains (which grew on nutrient agar). The numbers of cultivable As-resistant microorganisms recorded in the Arabian Gulf region on nutrient agar were similar to those of cultivable As-resistant bacteria in Lake Pontchartrain in Louisiana, USA, connected to the Gulf of Mexico (Jackson et al. 2005). However, the microbial taxa in the two ecosystems were, as expected, basically different, probably due to environmental variations. The only genera common to both habitats were *Agrobacterium* and *Bacillus*. The betaproteobacterium *Agrobacterium faecalis* has also been recorded by earlier investigators in other As- and Cd-contaminated sites (Jackson et al. 2005; Xiao et al. 2010). Actinobacteria belonging to the genera *Streptomyces, Nocardia*, and *Micromonospora* which were predominant As- (and Cd-) resistant inhabitants in the Kuwait desert soil samples as well as elsewhere (Kulkarni and Deshmukh 2001) were absent in lake Ponchartrain (Jackson et al. 2005). Other non-oily habitats harbor other As-tolerant actinobacteria (Routh et al. 2007), As-tolerant diazotrophic bacteria and betaproteobacteria (Oliveira et al. 2009) and species belonging to *Staphylococcus* and *Citrococcus* (Dave et al. 2010) as well as *Bacillus*, *Listeria*, *Achromobacter*, *Pseudomonas*, *Alcaligenes*, *Moraxella*, and *Planococcus* (Jackson et al. 2003; Salam et al. 2009). As far as cadmium is concerned, it has been recognized since decades that soil "actinomycetes" (viz *Nocardia, Micromonospora, Streptomyces*) were more tolerant to this metal than were the "eubacteria", and that gram-negative "eubacteria" (viz *Alcaligenes, Agrobacterium, Chromobacterium, Enterobacter, Proteus, Rhizobium*) were more tolerant than were gram-positive bacteria (viz *Bacillus, Brevibacterium, Corynebacterium, Micrococcus*; Babich and Stotzky 1977). Recent reports indicate that halophilic (10% NaCl) Cd-resistant bacteria from the Dead Sea could grow at 45°C in a medium containing 1,000 ppm of Cd (Massadeh et al. 2005). These literature reports show that bacterial taxa resistant to As were the same as those resistant to Cd, as our study also revealed.

The core and novel finding of the current study is that there are naturally in soil indigenous bacteria with the combined potential for As and Cd resistance and hydrocarbon utilization. In this context, our group in Kuwait has recently reported on indigenous bacteria with the combined potential for mercury resistance and -volatilization and hydrocarbon utilization in soils and plant rhizospheres (Sorkhoh et al. 2010a, b; Al-Mailem et al. 2010a, b). As in these last two studies on mercury, increasing As and Cd concentrations inhibited the hydrocarbon utilization potential by the individual microorganisms. However, within reasonably high concentrations of sodium arsenate (up to 40 mM) and cadmium sulfate (up to 0.9 mM) the individual strains of bacteria could consume at least a minimum of about 20% of the available crude oil in 2 weeks, which is certainly a satisfactory rate. In this context, Sizova et al. (2010) reported on two rhizospheric *Pseudomonas* spp. that oxidized naphthalene in the presence of As, and Xiao et al.

(2010) found that Cd contaminations of 0.01 mM, 0.1 mM and 0.5 mM did not affect the biodegradation of phenanthrene by *A. faecalis* (also recorded in this study) and *Brevindimonas* sp.

Interestingly, the highest oil consumption value was reached in the presence of 20 mM sodium arsenate (and, with some strains, 0.3 mM cadmium sulfate). In other words, rather low As (and in part Cd) concentrations could enhance oil bioremediation. Arsenate is biologically toxic in part through acting as a phosphate analog, thus interfering with phosphate uptake and transport (Tamaki and Frankenberger 1992). However, some bacteria may benefit from exposure to arsenate (Jackson et al. 2003) either by using it as a terminal electron acceptor in anaerobic respiration (Ahmann et al. 1994; Stolz and Oremland 1999; Oremland and Stolz 2003) or generating energy through chemoautotrophic arsenite oxidation (Santini et al. 2000). Arsenic seems to be metabolized by prokaryotes, participating in several functions viz. assimilation, methylation, detoxification, anaerobic respiration, and others (Stolz et al. 2006). These facts are related to the result that low arsenic concentrations enhanced hydrocarbon-consumption by all our isolates.

5 Conclusions

The core and novel finding of this paper is that the oil-free and oil-contaminated desert soil samples in Kuwait, probably elsewhere too, naturally contain appreciable numbers (10^3–10^5 CFU g^{-1}) of bacteria with the combined potential for As and Cdresistance and hydrocarbon utilization. Those strains utilize a wide range of individual alkanes and polynuclear aromatic hydrocarbons as sole sources of carbon and energy, which reflects a rather powerful hydrocarbon-bioremediation potential. This potential which was consolidated by the results of the quantitative GLC analysis of oil- and pure hydrocarbon consumption was maintained by the individual strains in the presence of a rather wide range of As and Cd concentrations. Those findings, in addition to the fact that low concentrations of both heavy metals enhance the microbial potential for hydrocarbon consumption make this group of indigenous soil bacteria valuable biological tools for self-cleaning and bioremediation of oily soils.

6 Recommendation and perspectives

For bioremediation of soil co-contaminated with hydrocarbons and heavy metals, microorganisms with the combined potential for hydrocarbon utilization and heavy metal resistance should be used to enhance hydrocarbon biodegradation in soil. The results of this study show that there is no need for molecularly "designing" microorganisms with these combined activities to inoculate them into the polluted sites. The latter already harbor appreciable numbers of such microorganisms which can immediately start the bioremediation process. Although higher concentrations of As and Cd inhibit the microbial potential for hydrocarbon consumption, low concentrations especially of As, stimulated this activity. Based on these results, it may be recommended to determine As in the oil-polluted sites prior to the bioremediation program, and to correspondingly dilute the As level to less than 20 mM arsenate, using pristine soil. Alternatively or in addition, oil bioremediation could be stimulated by the addition of simple organic compounds (Radwan et al. 2000)

Acknowledgments This work has been supported by the Kuwait University, Research Grant SL 08/07. Thanks are due to the SAF unit and GRF, Kuwait University for their help in GLC, (GS 02/01), ICP-MS (GS 01/05), and Genetic analyzer (GS 01/02).

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