Detection of plasmids in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas

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Abstract

Several heavy metal resistant bacterial strains were isolated from sediment and water samples collected from the Persian Gulf and enclosed industrial areas. All the isolated bacteria were identified by 16S rRNA gene sequencing. Isolated bacteria were tested for the presence of plasmids using the modified alkaline lysate method. The method was effective for identification and characterization of plasmids of different sizes without the use of highly toxic chemicals. The study revealed that the frequency of the occurrence of plasmids in heavy metal resistant bacteria was more than that in the common bacteria. The study also demonstrated that about 66% of isolated bacteria carried large (38-62kb) and/or small sized (4- >2 kb) plasmids. The highest plasmid incidence (84.6%) was detected from industrial wastewater bacteria. A slightly higher incidence of plasmids occurred in bacteria isolated from marine sediments (55.5%) compared to that of the marine water (53.8%). The findings suggested that plasmids are highly ubiquitous and predominant in most heavy metal resistant bacteria. Removal of lead and cadmium from solution by some of these bacteria was very efficient, approximately 120 mg/g dry weight-as high as 90%. The isolates tested, presented distinct uptake capacities and the best results were obtained for *Delftia tsuruhatensis* and *Pseudomonas* AU3411 respectively.

Keywords: Coastal zone; Persian Gulf; Industrial areas; Plasmid; Heavy metal resistance bacteria.

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INTRODUCTION

Plasmids are extrachromosomal genetic elements that can range in size from several hundred base pairs to several thousand kilobases (Cook *et al*., 2001). Plasmid-encoded genes represent a pool of mobile DNA that contributes significantly to the adaptation of natural microbial communities. An example of such plasmid-mediated adaptation has been reported previously for microbial populations during periods of pollutional stress when the frequency of catabolic plasmids increased by as much as 2- to 10-fold in polluted marine and freshwater ecosystems (Burton *et al*., 1982). Numerous studies have reported on the incidence of bacterial plasmids in marine sediments and estuarine and pelagic ecosystems (Belliveau *et al*., 1991). Marine plasmids with heavy metal resistance traits obtained either through exogenous (Dahlberg *et al*., 1997) or endogenous isolation methods, have been shown to exhibit broad host ranges (Sobecky *et al*., 1998), to be mobilizable (Beeson *et al*., 2002) and have self-transfer capabilities (Sandaa and Enger, 1994; Dahlberg *et al*., 1998).

Recent efforts have focused on characterization at the molecular level, plasmid populations and associated mobile genetic elements occurring in a wider range of aquatic and terrestrial habitats. Surprisingly, little information is available regarding the incidence and distribution of plasmids in contaminated subsurface environments. Such studies will provide greater knowledge on the ecology of plasmids and their contributions to the genetic adaptation of naturally occurring subsurface

microbial communities (Coombs and Barkay, 2004).

Microbial species, such as *Pseudomonas*, have been shown to be relatively efficient in bioaccumulation of uranium, copper, lead, and other metal ions from polluted effluents (Mullen *et al*., 1989; Gupta *et al*., 2001). Bacterial resistance to heavy metals is widespread. In one investigation, twenty-five heavy metal resistance strains were isolated from the Alzahra Hospital of Isfahan in Iran. Fifteen of the 25 isolates from nosocomial infections were identified as *Escherichia coli*, and remaining as *Kelebsiella pneumoniae*. Most of the isolated bacteria demonstrated multiple resistances to four heavy metals (Hg, Cu, Pb and Cd). In most of the 25 isolated strains, plasmids of different molecular size were observed **(**Karbasizaed *et al*., 2003).

The uptake of heavy metals, present in industrial wastes, and detoxification of metal ions by bacteria provide an additional mechanism of environmental bioremediation. In the present study the isolation of heavy metal resistant bacteria from the Persian Gulf and enclosed industrial areas were studied for the presence of plasmids. The data obtained shall provide clues regarding the prevalence of plasmids in heavy metal resistant bacterial populations residing in the marine and industrial areas. This could be used as an indicator to determine the degree of bacterial activity in eliminating toxic metals from the environment. The present study deals with isolation and characterization of plasmids in the heavy metal resistant bacteria isolated from the Persian Gulf and enclosed industrial areas.

MATERIALS AND METHODS

Sampling: Samples were collected from 12 transects at 72 stations, which were marked from 1 to 72, covering the whole Persian Gulf area (Fig. 1). Sample collections started from the Oman Sea and finished near Kuwait. Water samples were collected by niskin bottles, which were sterilized by 75% ethanol prior to water collection and washed with seawater samples were collected by niskin bottles into sterilize 250 ml of glass bottles. Sediments were collected by sterilize Van Veen Grab into sterilize plastic bags.

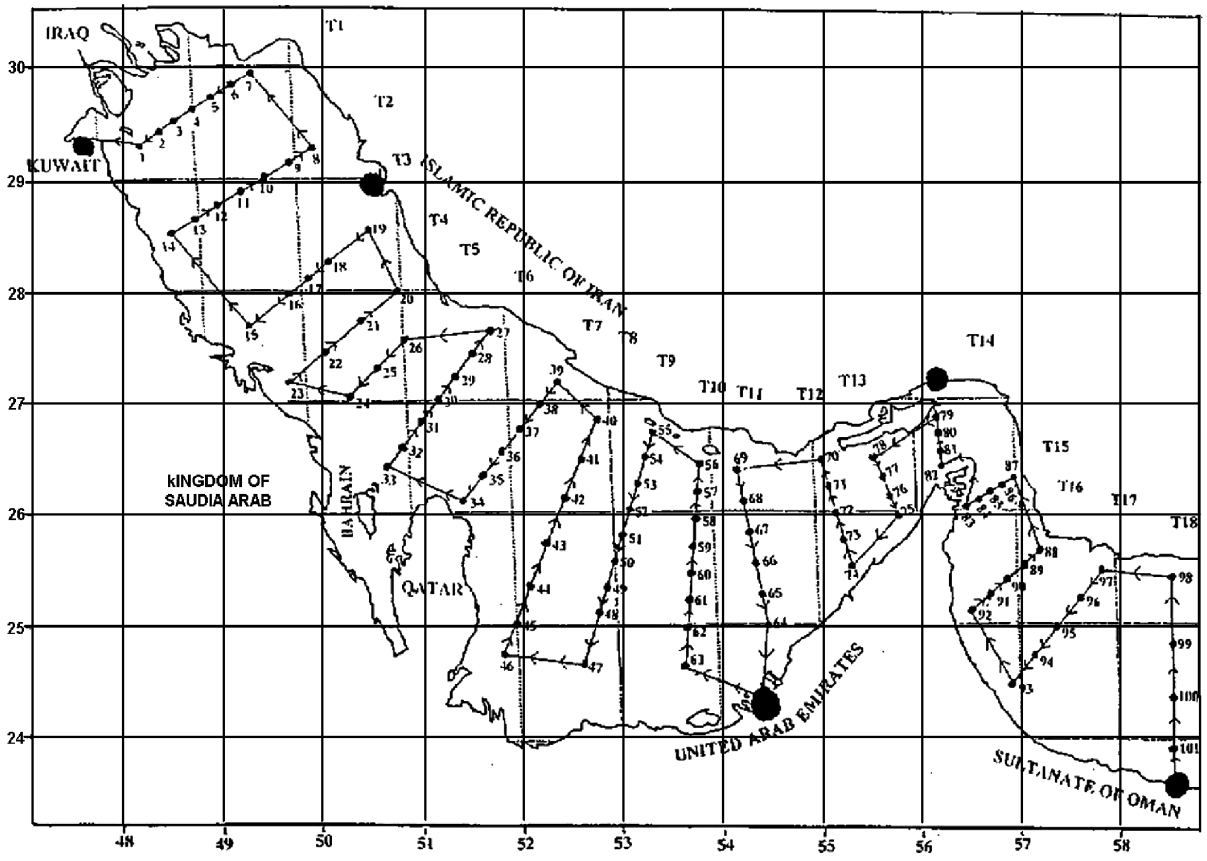

Figure 1. Sampling location including: transects (T_1-T_{13}) and stations (1-72) in the Persian Gulf on summer 2001, the cruise started from Oman sea and finished near Kuwait. The samples were collected from 72 stations of 15 transects of water and sediment.

Isolation of heavy metal-resistant bacteria: The water samples were inoculated on nutrient agar plates, containing 1 mM Cd ($Cd(NO_3)_24H_20$), 1 mM Cu($CuSO_45H_20$), 1 mM Zn ($ZnSO_47H_2O$) and 1 mM Pb ($Pb(NO_3)_2$. Plates were incubated at 28ºC for 72 hr. The sediment samples were diluted 10-1000 folds and inoculated in the same way. The resulting colonies were examined for heavy metal uptake ability and plasmid incidence.

Identification of bacterial isolates: All isolated bacteria were identified by sequences of 16S rRNA genes. Genomic DNA was extracted from isolated bacteria using a commercial kit (Bactozol®), according to the manufacturer's instructions. The two universal oligonucleotide primers used to amplify the 16SrRNA samples were as follows: forward primer, 5′-CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3′; and reverse primer, 5′-CCCGGGATCCAAGCTTACGG CTACCTTGT TACGACTT-3′. They were used in the amplification of 16S rRNA genes in all the isolated bacterial strains. PCR amplification was performed in a total volume of 100 ul mixture, containing 10 ul chromosomal DNA (100 ng) , 5 ul of 16S forward primer (20 pmol), 5 ul of 16S reverse primer (20 pmol), 4 ul of 5 mM 4 dNTP mix, 4 ul of 50 mM MgCl2, 10 ul of l0x *Taq* Buffer (supplied with the *Taq* DNA polymerase) , 61.5 ul sterile distilled water, 0.5 ul (2.5U) *Taq* DNA polymerase, mixed, and microcentrifuged briefly. The tubes were subjected to 30 cycle in a thermal cycler with the following program: 2 min at 95°C (to denatures the DNA and primers), 30 cycles of 30 sec. at 45°C, 2 min at 72°C and 30 sec. at 95°C, 1 cycle of: 1 min at 45°C, 2 min at 72°C. All samples were removed from the thermal cycler and stored at -20°C Electrophoresis of the PCR product from the 16S rRNA gene was carried out in 1% low melting agarose gel for 1 h. The gel was observed and precisely cut under UV with in a short time of less than 15 seconds. Then, 200 mg of the excised gel was transferred to a clean 1.5 ml microcentrifuge tube using a commercial Genomic DNA purification kit (Geni, pin USA), according to the manufacturer's instructions. The clean PCR product was subjected to cycle sequencing in both directions using universal primers. The sequencing was carried out by using the ABI PRISM Dye terminator cycle sequencing method (Perkin Elmer). Sequencing reactions were setup according to the instructions manual. The obtained nucleotide sequences were edited using the softwares Chromas and Bioedit and compared to published sequences in the NCBI GenBank.

Medium preparation and growth condition: All bacterial isolates were grown in 500 ml flasks of Terrific broth (TE) broth medium containing: 12 g of bacto-tryptone, 24 g of yeast extract, 4 ml of glycerol, and 900 ml of distilled water. The medium was supplemented with four metal salts, comprise 1 mM Cd ($Cd(NO_3)_24H_20$), 1 mM Cu($CuSO_45H_20$), 1 mM Zn ($ZnSO_47H_2O$) and 1 mM Pb ($Pb(NO_3)_2$ in the flasks and was autoclaved for 20 min. After cooling, 100 ml of filter sterilized phosphate stock solution was added to final volume of 1000 ml.

The bacterial strains used for plasmid isolation in this study are listed in Table 1. The bacteria isolated from industrial waste and marine water were directly inoculated on to nutrient agar plates. Bacteria isolated from marine sediments and sludge were serially diluted (1g) in artificial seawater and distilled water, respectively. The samples were spread onto solid media (nutrient agar) and plates were incubated for 1 to 14 days at 28°C. Colonies were picked from the plates and re-streaked at least twice on the same medium to ensure purity.

Plasmid isolation: The presence of plasmids in the marine and industrial bacterial isolates was determined using a modification of alkaline lysate method (Brinboim and Doly, 1979; Surzycki 2000). In the modified method the alkaline lysate was neutralized with ammonium acetate rather than potassium or sodium salts. This procedure allows purification of plasmid DNA without the use of toxic organic solvents, CsCl centrifugation or column chromatography.

Bacterial isolates were inoculated into 500 ml flasks that contained 30 ml of terrific broth medium supplemented with the appropriate four metal salts, and grown overnight at 28°C with vigorous shaking at 250 rpm. Culture samples were then transferred into 50 ml centrifuge tubes, and centrifuged at 4000 ×*g* for 5 min at 4°C. Cell pellets were then re-suspended gently in 3 ml of solution I which contained; 200 mM NaOH and 1% of SDS (W/V). A 100 µl of freshly-prepared lysozyme stock solution was added to the tube and mixed by vortexing, then incubated for 20 min at room temperature. After incubation, 6 ml of the cloudy solution II which contained; 25 mM Tris HCl, 10 mM

Table 1. Identification of bacteria isolated from Persian Gulf and enclosed industrials.

Strain No	Identification	Accession No	Location of isolation	Estimated plasmid size (Kb)	Identification - on station
1	*Pseudomonas sp. M18*	AY394845	coast industrial	56 kb, 2 kb and <2kb	coast zone
2	*Delftia acidovorans* strain 30V3	AF526915	Sea water	60 Kb	coast zone
3	*Brevundimonas diminuta*	DQ857897	coast industrial	unknown	coast zone
4	*Pseudomonas. Aeroginosa* ATCC 27853	D 89652.	urban waste water	52 Kb, 2 Kb and <2Kb	coast zone
5	*Delftia sp. AN3*	AY052781	Sea water	52 Kb, 2 Kb and <2Kb	15
6	*Pseudomonas sp. K2*	AF532866	sediment	52 Kb, 2 Kb and <2Kb	12
7	*Pseudomonas sp. ZJF08*	EF660438	coast industrial	52 Kb, 10Kb, 2 Kb and <2Kb	coast zone
8	*Delftia. tsuruhatensis*	AJ606337	Sea water	60 Kb	40
9	*Pseudomonas. Putida* MM1	AY623928	coast indust rial	52 Kb, 2 Kb and <2Kb	coast zone
10	*Pseudomonas aeroginosa* ATCC 27853	D 89652.	coast industrial	56 Kb,4 Kb 3Kb, 2 Kb and <2 Kb	coast zone
11	*Pseudomonas aeroginosa* ATCC 27853	D 89652.	coast industrial	68 Kb and <2 Kb	coast zone
12	*Methlobacteru m sp. Mil*	AY436803	Sea water	unknown	17
13	*Peudomonas sp. K2*	AF532866	coast industrial	68 Kb and <2 Kb	coast zone
14	*Pseudomonas tolaasii*	AF255336	coast industrial	42 Kb, 2 Kb and <2 Kb	coast zone
15	*Pseudomonas aeroginosa* ATCC 27853	D 89652.	coast industrial	42 Kb, 2 Kb and <2 Kb	coast zone
16	*Pseudomonas sp. Fa27*	AY131221	coast industrial	52 Kb, 2 Kb and <2Kb	coast zone
17	*Pseudomonas sp. M18*	AY394845	sediment	38 kb, 16 kb, 2 kb and <2 kb	1
18	*Pseudomonas aeruginosa* strain AU4594	AY486367	sediment sample	unknown	25
19	*Bacillus pumilus strain* DSMZ27	AY456263	sediment	unknown	12
20	*Arthrobacter sp. Fa21*	AY131225	Sea water	unknown	32
21	*Comamonas terrigena*	AJ430343	sediment sample	42 kb	26
22	*Pseudomonas aeruginosa* BHP7-6	AY162139	coast industrial	unknown	coast zone
23	*Methylobacterium sp* . (strain F18)	D32233	sediment	unknown	11
24	*Methylobacterium mesophilicum*	AJ400919	sediment	unknown	12
25	*Bacterium Ph10*	AY345364	sediment	62 kb, 2 kb and <2 kb	14
26	*Dechlorosoma sp. PCC*	AY126453	sediment	unknown	12
27	*Staphylococcus sp. LMG 21006*	AJ316320	sediment	unknown	6
28	*Pseudomonas putida 269*	EF615010	sediment	62 kb, 2 kb and <2 kb	12
29	*Pseudomonas nitroreducens*		Sea water	unknown	24
30	*Pseudomonas sp. K2*	AF532866	Sea water	62 kb, 2 kb and <2 kb	17
31	*Pseudomonas sp. Fa27*	AY131221	Sea water	unknown	40
32	*Bacterium RRP -E3*	AJ536683	sediment	62 kb and <2 kb	16
33	*Delftia tsuruhatensis*	AJ606337	sediment	2 kb and <2 kb	26
34	*Delftia tsuruhatensis*	AJ606337	Sea wa ter	2 kb and <2 kb	coast zone
35	*Pseudomonas sp. IC038*	U85869	coast industrial	2 kb and <2 kb	coast zone

Na_2EDTA (pH 8.5) and 0.9% glucose (W/V) (placing solution II on ice until become cloudy), was added. The tube was mixed by inverting it 6 times and then incubating it on ice for 10 min. A 4.5 ml sample of ice cold 7.5 M ammonium acetate solution was added to the tube and gently mixed by inverting it 6 times. It was then incubated on ice for an additional 10 min, and then centrifuged in a swinging bucket rotor at 18,000 $\times g$ for 10 min at 4°C. The supernatant was then transferred into a 50 ml centrifuge tube. 8.1 ml of isopropanol was added to the supernatant and mixed by inverting the tube several times before incubating for 10 min at room temperature. After incubation, the solution was centrifuged at 18,000 $\times g$ for 10 min after which alcohol was removed. Six ml of 1. 87 M ammonium acetate solution was added to the tube and mixed by vortexing, and then incubated on ice for 10 min. The resulting sample was centrifuged at 18,000 $\times g$ for 5 min at room temperature and the supernatant transferred into a fresh 50 ml tube. Twelve ml of 95% ethanol (v/v) was added into the tube and mixed well by inverting the tube several times. The tube contents were then centrifuged at 18,000 $\times g$ for 15 min at room temperature and the supernatant discarded. Seventy percent (70%) of cold ethanol (v/v)was added slowly to the tube that was kept at a 45° angle, and the alcohol in the mixture was immediately discarded. This washing step was repeated twice. After brief centrifugation, the remaining ethanol was collected with a capillary tip. Plasmid pellets were dissolved in 300 µl of TE buffer and transferred to 1.5 ml micro-fuge tubes. 5 µl of RNase A and 1 µl of RNase T1 were added to each tube, mixed well and then incubated in a 37°C water bath for 30 min. After incubation, 150 µl of 7.5 M ammonium acetate was added to the tube and mixed by inverting 5 times, 900 µl of 95% ethanol (v/v) was again added to the tube by following mixing well. The tube was centrifuged at at 18,000 $\times g$ for 10 min at room temperature and the ethanol was then removed gently. The resulting plasmid pellets were washed with 700 µl of cold 70% ethanol (v/v) three times, the tubes were stored in an inverted position for two min. Finally 30 µl of TE buffer was gently added to the tube to dissolve plasmid DNA.

Determination of DNA concentration: Plasmid DNA was diluted in phosphate-buffered saline (PBS) (1:100), and the concentration and purity of the extracted plasmids were determined spectrophotometrically (Pe´rez-Luz *et al*., 2004). The optical density (OD) of the DNA was measured at 260 and 280 nm. The OD_{260} allowed calculation of the DNA concentration in the sample, where an OD_{260} of 1 corresponds to approximately 50 µg/ml of double stranded DNA. The ratio of the OD_{260} nm OD_{280} provides an estimate for the purity of the DNA.

Gel electrophoresis: Plasmid DNA was separated by electrophoresis on a 0.7% agarose gel (w/v) at 50 volts overnight. *E. coli* V517 plasmids were used in each gel as molecular markers. The gel was stained with ethidium-bromide, visualized under UV transillumination and photographed.

RESULTS

A total of 35 bacterial strains resistant to heavy metals were isolated from samples belonging to the marine environment and enclosed industrial areas. All of the strains were resistant to all four metals tested in this study (Cu, Pb, Cd, and Zn).

Preliminary identification indicated that most bacteria were Gram-negative and some of them produced pigments when grown in solid media. The 16S rRNA analysis revealed that all isolates belonged to the genera *Pseudomonas, Delftia, Brevundimonas, Methylobacterium, Bacillus, Arthrobacter, Staphylococcus* and the *Bacterium Ph10* species (Table 1). Sequences from 23 isolates had a similarity equal or higher than 95% with other 16S rRNA sequences from the database, while two had less than 90% similarity and the remaining were between 90 and 95%. Only *Pseudomonas* sp. Fa27 showed 99% similarity.

Out of the 35 bacterial tested for the presence of plasmids, 23 (66%) isolates showed plasmid DNA bands on the agarose gel (Figs 2A and 2B). Among the 23 plasmid containing bacterial strains, 15 strains (65%) belonged to *Pseudomonas* groups and 8 strains (35%) were identified as *Delftia acidovorans* 30V3, *Delftia* sp. AN3*, Delftia Tsuruhatensis, Methylobacterium* sp. Mil*, Bacillus pumilus* DSMZ27, *Arthrobacter* sp. Fa21*, Comamonas terrigena, Methylobacterium* sp. (strain F18), *Methylobacterium mesophilicum, Bacterium* Ph10*, Dechlorosoma* sp. PCC*, Staphylococcus* sp. LMG 2100 *and Bacterium* RRP-E3.

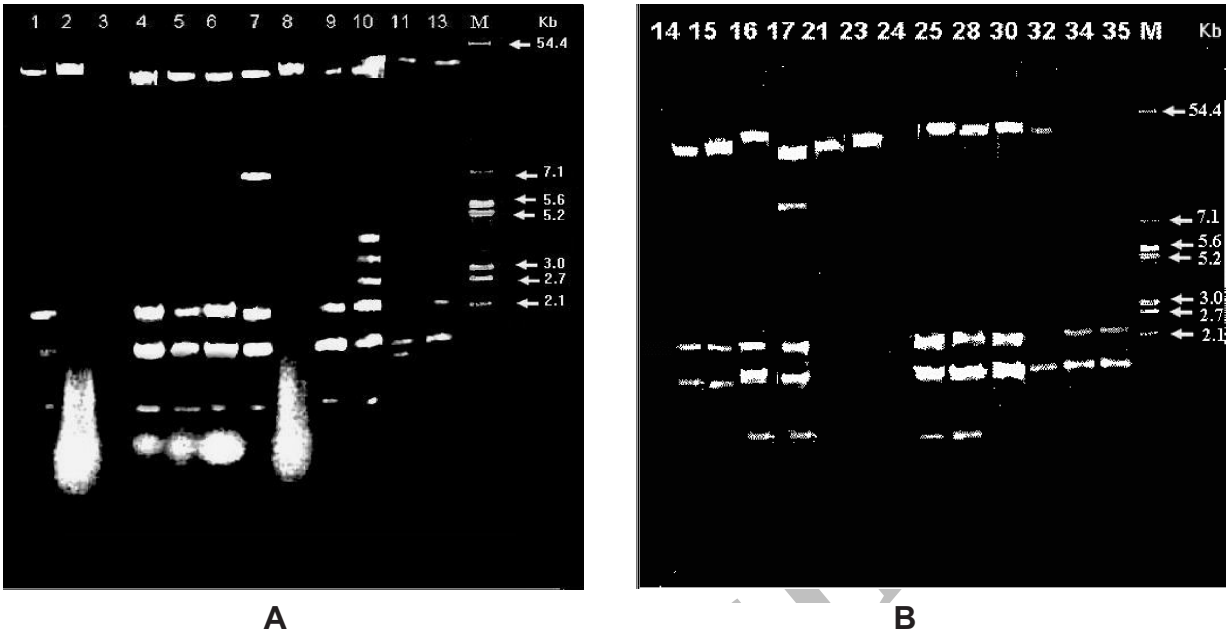

Figure 2. Ethidium bromide-stained 0.7% agarose gel electrophoresis of representative plasmids isolated by new the modified alkaline lysate method from isolates bacteria, A (lanes 1 to 13) with Marker E. coli V517 and B (lanes 14 to 35) with Marker *E. coli*. V517.

Bacterial isolated from industrial waste-water had the highest plasmid incidence 48% followed by sediment 30%, and those from seawater had the lowest plasmid incidence 22% (Table 2). Bacterial isolated from two different environments; marine and industrial waste-water, as the second one exhibited more frequency of plasmid incidence.

The relative migration of unknown plasmids were estimated by the use of plasmid molecular weight marker, the size of the plasmids also were calculated by labimage software. Twenty bacteria strains have plasmids with a molecular weight large sizes between 38 to 62 kb, Two strains had plasmids with a molecular weight between 10 kb and 16 kb, 20 strains had plasmids with 4 kb molecular weight and smaller than 4 kb and also 17 isolates represented large plasmid size and small plasmid size contemporaneously (Table 2). The occurrence of plasmids according to sizes was illustrated as 20 strains had large plasmid size, 2 medium plasmid size, 20 small plasmid size and 20 large-small plasmid sizes contemporaneously (Table 2). Most of the heavy metals resistance bacteria were found to carrying plasmid. The difference between the marine and industrial waste water strains was most clearly seen in plasmid carrying, as the frequency of plasmid carrying bacteria among industrial waste water was found only in Pseudomonas strains. Most plasmid carrying bacteria in the marine strains were obtained from Delftia strains that carry plasmids of big and small sizes (Table 1).

DISCUSSION

Plasmid-encoded genes represent a considerable pool of mobile DNA that can contribute to the genetic adaptation of microbial communities. Plasmid containing of the isolated marine bacteria have been examined by two methods, plasmid has been detected by exogenous isolation methods, which requires the initial isolation of bacteria to test for the presence of plasmids

Table 2. Molecular weight of the bacterial plasmids.

Plasmid size	*Pseudomonas*	Others	No. of plasmids	Frequency (%)
> 38 kb large	14	6	20	32.25
10-16 kb medium	2	0	2	3.25
4- <4 kb small	15	5	20	32.25
>38 and <2	15	5	20	32.25

Table 3. Frequency of plasmid-carrying by isolated from different sample sources.

Sampling area	Source of strains	No of bacterial isolates	No of strains with plasmid	*Pseudomonas*	Other groups	% of strains with plasmid
Industrial areas	Waste water	13	11	11	0	48%
Persian Gulf	Sea water	9	5	1	4	22%
Persian Gulf	Sediment	13	7	3	4	30%

(Dahlberg *et al*., 1997). However, many plasmids occurring in marine sediment bacterial populations are also identified by endogenous isolation techniques which does not require the isolation of the plasmid-bearing host bacterium but instead relies primarily on the ability of natural plasmids to transfer to a selected recipient (Sobecky *et al*., 1997).

The presence of plasmids in marine sediment and water-column bacteria is well documented (Aviles *et al*., 1993; Dahlberg *et al*., 1997; Ghosh *et al*., 2000). Plasmids in natural populations of marine bacteria have been reported to have the following frequencies: 23% in an unpolluted site of the Gulf of Mexico, 46% in Chesapeake Bay, 43% (marine luminous bacteria) in the Mediterranean and Red sea, and 28% in the Antarctic (Kobori *et al*., 1984).

In the present study all the 35 isolated bacterial strains were tested for the presence of plasmid using the modified alkaline lysate method. The isolated plasmids were divided into three sizes; small, medium and large, having molecular weights of approximately 4-2 kb, 10-16 kb and 38-62 kb, respectively. The frequencies of plasmid-carrying isolates from heavy metal resistant bacteria were as 32.25% with sizes of 4- 2 kb, 3.25% with sizes of 10 and 16 kb and 32.25% with sizes of 38-62 kb.

An example of plasmid-mediated adaptation has been reported previously for microbial populations during periods of pollutional stress when the frequency of plasmid occurring bacteria was found to increase by as much as 2 to 10 fold (Sobecky, *et al*., 1997). Therefore, it is expected that the frequency of plasmids in heavy metal resistant bacteria to be higher than that in natural bacteria community (Kobori *et al*., 1984). Plasmids have been observed with an incidence of 3% in strains from agricultural soils as compared to the 42% incidence observed in strains from industrial soils (Campbell *et al*., 1995). The results of this study showed a higher plasmid incidence in the isolated bacteria (Table 3).

It was also shown that some isolated bacteria were capable of taking up heavy metals at high concentrations when exposed to 0.5 mM and 1mM Zn, Cu, Pb and Cd. For instance, when bacteria were exposed to Cu, maximum quantities of Cu was taken up by *Pseudomonas* SP.Fa27 at 54.2 mg per gram dry weight of cells. Maximum Pb removal was found to as high as 90% by *Pseudomonas* strain AU3411. After successive subculturing, there appeared to be significant differences in the ability of heavy metal accumulation by the plasmid containing bacteria. Some of the isolated bacteria lost their plasmids and so their ability to resist and accumulate heavy metals after a few subcultures. This result demonstrated that some of these bacteria obtained their heavy metal resistance trait through their plasmids. These isolated bacteria demonstrate that resistance to heavy metals by genes present on their plasmids suggests the exertion of selective pressure on such bacteria through contamination with heavy metals in their environment. The elevated rate of resistance to metals reflects an adaptive response to the presence of toxic elements in different environments such as water, sewage, rivers, and estuaries. Heavy metal resistance genes are often found on plasmids and transposons (Chu *et al*., 1992; Silver, 1992). The marine and industrial waste water environments have exposed bacteria to large amounts of heavy metals. This contamination has taken place over several decades, so that the current bacterial populations are well adapted to such environmental pollutants. When the bacteria from industrial area samples were compared to those from marine environment there were clear difference. Some areas of the marine environment were found to demonstrate the presence of heavy metal bacteria.

The genus of Pseudomonas comprises a vast and rather diverse group of bacteria, they can be found in a variety of natural environments. The extent of the prevalent of plasmids in pseudomonas species inhabits them in specific natural environment. Plasmids also assist to bacteria to acquire tolerance and resistance mechanisms against heavy metals or other toxic substances in the pollutant environment. For instance, the

incidence of plasmids in Pseudomonas strains was significantly higher in pollutant water strains than in the same bacteria isolated from non pollutant water (Boronin 1992). The present study is in agreement with those reported by Boronin (1992). The present study demonstrated about 66% of isolated bacteria had plasmid. In contrast, there was as little as 3% of the incidence in the strains from agricultural soils and 42% incidence in the strains from industrial soils (Campbell *et al*., 1995). Among 19 Pseudomonas strains isolated from marine and closed industrial waste water 15 strains were found to contain plasmid in different sizes. This study also appeared plasmids incidence in Pseudomonas strains were more frequent than other strains (Table 2).

CONCLUSION

It was also observed that the frequency of plasmids in the heavy metal resistant bacteria are higher than that of the natural bacteria.

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