Malaysian Journal of Microbiology, Vol 4(2) 2008, pp. 42- 50
http://dx.doi.org/10.21161/mjm.11808

Isolation, characterization and application of bacterial population from agricultural soil at Sohag Province, Egypt

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Received 18 June 2008; received in revised form 16 October 2008; accepted 16 October 2008

ABSTRACT

Forty soil samples of agriculture soil were collected from two different sites in Sohag province, Egypt, during hot and cold seasons. Twenty samples were from soil irrigated with canal water (site A) and twenty samples were from soil irrigated with wastewater (site B). This study aimed to compare the incidence of plasmids in bacteria isolated from soil and to investigate the occurrence of metal and antibiotic resistance bacteria, and consequently to select the potential application of these bacteria in bioremediation. The total bacterial count (CFU/gm) in site (B) was higher than that in site (A). Moreover, the CFU values in summer were higher than those values in winter at both sites. A total of 771 bacterial isolates were characterized as *Bacillus, Micrococcus, Staphylococcus, Pseudomonas, Eschershia, Shigella, Xanthomonas, Acetobacter, Citrobacter, Enterobacter, Moraxella* and *Methylococcus.* Minimum inhibitory concentrations (MICs) of Pb^{+2}, Cu^{+2}, Zn^{+2}, Hg^{+2}, Co^{+2}, Cd^{+2}, Cr^{+3} Te^{+2}, As^{+2} and Ni^{+2} for plasmid-possessed bacteria were determined and the highest MICs were 1200 μg/mL for lead, 800 μg/mL for both Cobalt and Arsenate, 1200 μg/mL for Nickel, 1000 μg/ml for Copper and less than 600 μg/mL for other metals. Bacterial isolates from both sites A and B showed multiple heavy metal resistance. A total of 337 bacterial isolates contained plasmids and the incidence of plasmids was approximately 25-50% higher in bacteria isolated from site (B) than that from site (A). These isolates were resistance to different antibiotics. Approximately, 61% of the bacterial isolates were able to assimilate insecticide, carbaryl, as a sole source of carbon and energy. However, the *Citrobacter* AA101 showed the best growth on carbaryl.

Keywords: Plasmid incidence, heavy metals, CFU, agricultural soil, carbaryl, wastewater, antibiotics, SDS-PAGE

INTRODUCTION

Soil contains a variety of microorganisms included bacteria that can be found in any natural ecosystem. Microorganisms play an important role on nutritional chains that are an important part of the biological balance in the life in our planet. Where, bacteria are essential for the closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulfur and phosphorous cycle. Without bacteria, soil would not be fertile and organic matter such as straw or leaves would accumulate within a short time (Kummerer, 2004). Microorganisms can be used to determine the bioavailability of a given chemical compound in soil. Specifically, measurement of plasmid-containing bacteria, using either an endogenous or exogenous approach, serves as a general indicator of environmental contaminants (Arias *et al*., 2005). In the endogenous approach, plasmids are extracted from soil bacteria isolated on agar plates followed by a visualization of the plasmids on agarose gels (Campbell *et al*., 1995).

Soils normally contain low background levels of heavy metals. However, in areas where agricultural, industrial or municipal wastes are land-applied as fertilizer, concentrations may be much higher. Excessive levels of heavy metals can be hazardous to man, animals and plants. Although most organisms have detoxification abilities (i.e mineralization, transformation and/or immobilization of pollutants), particularly bacteria, play a crucial role in biogeochemical cycles and in sustainable development of the biosphere (Diaz, 2004).

Microbial survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Wuertz and Mergeay, 1997). For example, high levels of heavy metals can affect the qualitative as well as quantitative composition of microbial communities. Several studies have found that metals influence microorganisms by harmfully affecting their growth, morphology, and biochemical activities, resulting in decreased biomass and diversity (Baath, 1989; Reber; 1992; Malik and Ahmed, 2002). Previous studies have shown that long term (Hada and Sizemore, 1981; Duxbury and Bicknell, 1983) and short term (Hardman *et al*., 1986; Wickham *et al*., 1988) stresses such as high temperature, extremes of pH or chemical pollution often result in altered metabolism, species diversity and plasmid incidence of soil bacteria populations.

Some microbial strains possess genetic determinants that confer the resistance. In bacteria, these determinants are often found on plasmids, which have facilitated their study at the molecular level

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(Cervantes *et al*., 1994). Bacteria isolated from toxic chemical wastes more frequently contain plasmid DNA and demonstrate antimicrobial resistance than do bacterial isolates from domestic sewage-impacted waters or from uncontaminated open ocean sites (Baya *et al*., 1986). A higher incidence of plasmids was found among *pseudomonas*-like organisms in an industrially polluted river (18%) than in a non-polluted upstream area (7%) (Burton *et al*., 1982). If the number of plasmids is found to have increased at a given site, an investigation of the responsible stress factor can be initiated (Arias *et al*., 2005). Similarly, monitoring of antibiotic-resistant bacteria in soil can be used as an indicator of industrial and urban pollution.

This work was aimed to study the incidence of plasmids in bacteria isolated from agriculture soil irrigated with wastewater and canal water. The present work was also aimed to characterize these plasmids and to study the capability of the bacterial strains harboring these plasmids for degradation of different pesticides and resistance to a variety of antibiotics and heavy metals.

MATERIALS AND METHODS

Collection of soil samples

Forty samples of agricultural soil were collected from different localities at Sohag city which located at ~ 475 kilometers south from Cairo and ~ 400 kilometers north from Aswan. Twenty samples were taken from agriculture soil irrigated with canal water (site A) and twenty from agriculture soil irrigated with wastewater (site B), where sewage is used directly to irrigate the agriculture soil as a supplement of essential plant nutrients. Samples were collected during the period from February 2005 to January 2006. The soil samples were collected twice, one period from April to September (hot season) and another from October to March (cold season). Soil samples from soil surface (0-5 cm) and at a depth of approximately 20 cm (around the plants roots) were taken in sterilized polyethylene bags using sterilized spatula and stored at 4 ºC until examination.

Isolation and identification of bacteria

The soil samples were passed through a sieve (1.7 mm mesh) to remove large pieces of debris and vegetation. The bacteria were originally isolated by plating dilutions of soils in saline solution (0.9% NaCl) on nutrient agar and incubated at 37 ^{o}C for 48 h. The developed colonies were counted in plates and the average number of colonies per three plates was determined. The number of total bacteria (CFU) per gram dry weight soil was determined. Individual colonies of bacteria which varied in shape and color were picked up and purified by streaking on nutrient agar. The bacterial isolates were kept on nutrient agar at 4 °C and recultured every 4 weeks. The bacterial isolates were identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

Determination of MIC (Minimum Inhibitor Concentration)

Minimum inhibitory concentrations (MICs) of the metals were determined by the plate-dilution method as described by Malik and Jaiswal (2000). The metals Pb^{+2}, Cu^{+2}, Zn^{+2}, Hg^{+2}, Co^{+2}, Cd^{+2}, Cr^{+3} Te^{+2}, As^{+2} and Ni^{+2} were used as $Pb(NO_3)_2$, $CuSO_4$, $ZnSO_4$, $HgCl_2$, $CoCl_2.6H_2O$, $CdSO_4$,$K_2Cr_2O_7$, K_2TeO_3, $NiSO_4$, respectively. Stocks of the metal salts were prepared in distilled water and sterilized by filter membrane (0.22 μm) and stored at 4 °C. The heavy metal solutions were added to nutrient agar in various concentrations ranging from 3.5 to 3200 µg/mL and then spot inoculated with approximately 3 x 10^6 bacteria. The plates were incubated at 30-37 ºC for 48 hours. The concentration of the metal which permitted growth and beyond which there was no growth was considered as the MIC of the metal against the strain tested.

Biodegradation of insecticides

Bacterial isolates were grown in minimal salt medium (MSM) [0.5 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4.7H_2O$, 0.05 g $CaCl_2$, 2.44 g Na_2HPO_4 and 1.52 g KH_2PO_4, a liter distilled water, pH at 6.8] supplemented with 1 mM phenol, 50 µg/mL carbaryl or 50 µg/mL cypermethrin. The growth was at 37 ^{o}C in Gyratory shaker at 150 rpm.

Growth Kinetics of *Citrobacter* AA101 on carbaryl

Citrobacter AA101 was inoculated into 50 ml of MSM medium amended with 50 µg/mL cabaryl as a sole source of carbon and energy and incubated at 37 ^{o}C in Gyratory shaker at 150 rpm. The bacterial culture was collected at late log phase (~ 10^6/mL) and washed with phosphate buffer. A 100 µL of buffer-diluted cell suspension was subsequently transferred into 100 ml of MSM medium supplemented with carbaryl in triplicate conical flasks. A conical flask contained the MSM medium supplemented with 2% glucose using as a positive control and a conical flask contained only MSM medium was used as a negative control. The cultures were incubated at 30 ^{o}C in Gyratory shaker at 150 rpm. Growth was monitored at regular intervals by measuring the optical density (OD) at 600 nm.

Analytical methods

To detect the breakdown of carbaryl and the accumulation of degradation products, 1 mL samples of supernatant from growing culture of *Citrobacter* AA101 in MSM medium with carbaryl at zero time, 3 days, 6 days and 9 days from incubation at 37 ^{o}C were serially diluted and scanned at 200-600 nm with Perkin-Elmer Lambda 3 Spectrophotometer. The shift in the λ max indicates transformation of the compound (Tett *et al*., 1994).

Isolation of plasmid and electrophoresis

The bacterial isolates were screened for the presence of plasmid DNA using the lysis method (Sambrook *et al*., 2001) and the method described previously by Kado and Liu (Kado and Liu, 1981). The plasmid preparation samples were fractionated according to the method described by Sambrook *et al* (2001).

RESULTS

Total bacterial count in soil

The total bacterial count (CFU/gm soil) in site (A) fluctuated from 27 X 10^2 to 31 X 10^3 and from 8 X 10^2 to 23 X 10^3 during hot and cold seasons, respectively. However, the total bacterial count in site (B) ranged from 30 X 10^3 to 45 X 10^4 and from 13 X 10^2 to 30 X 10^3 during hot and cold seasons, respectively. These results indicated that the total bacterial count in hot season was higher than that in cold season in both two sites. Moreover, the total bacterial count values were different between both two sites during the same season.

During hot season, in site (A), the total bacterial count at surface area was 15.46 X 10^3 whereas 10.97 x 10^3 was recorded at the deep area. However, in cold season, 71.1 X 10^2 and 52.3 X 10^2 were recorded at surface area and deep area, respectively. At site (B), during hot season, the total bacterial count was 19.9 x 10^4 and 81 x 10^3 at surface and deep, respectively. However, in cold season, the total bacterial count was 12.1 x10^3 and 10.65 x10^3 at surface and deep, respectively. These results indicated that the culturable heterotrophic bacterial densities were usually higher at the surface than the depth at both two sites. Moreover, the density of total culturable bacteria in site (B) was significantly higher than those in site (A). The Bacterial density not only depends on the sample site but also on sample position.

Identification of bacterial isolates and their Frequency

A total of 771 strains were isolated from both two sites. 379 bacterial isolates were from site (A), and 392 isolates were from site (B). The bacterial isolates were purified and identified at the genus level by standard procedures described in Bergey's Manual of Systematic Bacteriology. Among Gram-positive bacteria, *Bacillus* was the most frequently genus isolated from site (A) and site (B) during hot and cold seasons. However, the genus *Bacillus* was more frequently isolated from soil irrigated with canal water. *Staphylococcus* and *Micrococcus* were variable recovered. *Micrococcus luteus* was only recovered from site (A) at frequency ~ 1.5% during cold season. However, *Staphylococcus* and *Micrococcus* were recovered from site (B) with different frequencies during hot and cold seasons.

Eight genera of Gram-negative bacteria were recorded at site (A). *Pseudomonas* was the most frequently isolated genus at frequency ~ 32% and 28% during hot and cold seasons, respectively. *Escherichia*, *Xanthomonas,* and *Enterobacter* were the second most frequent genera recorded at site (A). Where, *Escherichia* were recorded at frequency ~ 12% and ~ 8% during hot and cold seasons, respectively. Whereas, Xanthomonas and *Enterobacter* were recorded at frequency ~ 8% and ~ 12% during hot and cold seasons, respectively. *Acetobacter* was the third most frequent genus which was recorded at ~ 12% during cold season and at low frequency (~ 4%) during hot season. The remaining genera were less frequent either during hot and cold seasons. *Shigella* only recovered during cold season at frequency ~ 8% whereas *Moraxella* and *Methylococcus* were only recovered during hot season at frequency ~ 12% and ~ 8%, respectively.

At site (B), *Pseudomonas* was also the most frequently genus isolated during hot and cold seasons at ~ 28% and ~ 26%, respectively. *Escherichia* was the second most frequent genus recorded consistently at site (B) with frequency ~ 19% and ~ 14% during hot and cold seasons, respectively. *Shigella* was recorded at ~ 9% and ~ 12% during hot and cold seasons, respectively. Also, three genera namely *Xanthomonas*, *Acetobacter* and *Citrobacter* were recovered from site (B). *Xanthomonas* were recovered at ~ 7% during both hot and cold seasons whereas *Acetobacter* and *Citrobacter* were recovered at ~ 9% and ~ 4% during cold and hot seasons, respectively. *Citrobacter* was only recovered from site (B). In addition, *Enterobacter* and *Methylococcus* were recovered at variable frequency. *Enterobacter* and *Methylococcus* were recovered at frequency ~ 2% and ~ 9%, respectively during hot season and at the same frequency (~ 7%) during cold season.

Heavy metal resistance

Total of 771 bacterial isolates were tested for their resistance against different heavy metals such as Pb^{+2}, Cu^{+2}, Zn^{+2}, Hg^{+2}, Co^{+2}, Cd^{+2}, Cr^{+3}, Te^{+2}, As^{+2} and Ni^{+2} at different concentrations from 3.5 to 3200 μg/mL. Of the site (A), ~ 98% of isolates were resistant to 200 μg/mL Cu, ~ 41 % to 200 μg/mL Co, ~ 94 % to 600 μg/mL Pb, ~ 40 % to 400 μg/mL Zn, ~ 90 % to 400 μg/mL Ni , ~ 53 % to 50 μg/mL Cd, ~ 45 % to 27 μg/mL Te, ~ 43 % to 400 μg/mL As, ~ 63 % to 200 μg/mL Cr and ~ 34 % to 20 μg/mL Hg. However, in site (B), ~ 83 % of bacterial isolates were resistant to 200 μg/mL Cu, ~ 42 % to 200 μg/mL Co, ~ 100 % to 600 μg/mL Pb, ~ 32 % to 400 μg/mL Zn, ~ 100 % to 400 μg/mL Ni, ~ 27 % to 50 μg/mL Cd, ~ 37 % to 27 μg/mL Te, ~ 48 % to 400 μg/mL As, ~ 89 % to 400 μg/mL Cr and ~ 25 % to 20 μg/mL Hg. The highest MICs observed were 1200 μg/mL for Lead, 800 μg/mL for Cobalt and Aarsenate, 1200 μg/mL for Nickel, 1000 μg/mL for Copper and less than 600 μg/mL for other metals. These results indicated that high percentage of heavy metal resistance bacteria among the isolated strains recovered from soil irrigated with

wastewater (site B) compared to the bacterial strains isolated from soil irrigated with the canal water (site A).

The majority of the isolates from site (A) showed multiple resistances to metal ions. Approximately 84% of the isolates were exhibited resistance to the combination of two metals (300 µg/mL Ni and Pb) and ~ 80% of the isolates were exhibited resistance to (300 µg/mL Ni and Cu). Moreover, ~ 11% of the isolates were exhibited resistance to the combination of three metals (500 µg/mL Cu, 50 µg/mL Cd and 600 µg/mL Pb), ~ 7% of the isolates were resistant to (300 µg/mL Cu, 400 µg/mL Ni and 400 µg/mL Pb) and ~ 37% of the isolates were resistant to (200 µg/mL Zn, 30 µg/mL Cd and 300 µg/mL Pb). Furthermore, ~ 4% of the isolates were resistant to the combination of four metals (500 µg/mL Cu, 600 µg/mL Ni and Pb, and 200 µg/mL Zn).

At site (B), ~ 81 % of the isolates were resistant to the combination of two metals (300 µg/mL Ni and Pb) and ~ 79% of the isolates were resistant to (300 µg/mLed Ni and Cu). Moreover, ~ 4% of the isolates were resistant to (300 µg/mL Cu, 400 µg/mL Ni and Pb) and ~ 21% of the isolates were resistant to (200 µg/mL Zn, 30 µg/mL Cd and 300 µg/mL Pb). However, ~ 1 % of the isolates were resistant to the combination of four metals (500 µg/mL Cu, 600 µg/mL Ni and Pb, and 200 µg/mL Zn) and ~1% of the isolates were resistant to combination of five metals (200 µg/mL Co and Cr, 300 µg/mL Ni and Pb and 200 µg/mL Cu).

Incidence of bacterial isolates possessed plasmids

All 771 bacterial isolates recovered from soil samples were screened for the presence of plasmids. Figure 1 demonstrated the agarose gel electrophoretic patterns of plasmids from nine multiple metal and antibiotic resistance bacteria. Plasmids were found only in 337 bacterial isolates. Approximately 138 bacterial isolates from site (A) and ~ 199 isolates from site (B). The results showed that bacterial isolates taken from site (B) had the highest plasmid incidence of ~ 51% and ~50% in hot and cold seasons, respectively. However, the bacterial isolates from site (A) had the lowest plasmid incidence of ~ 36% during both hot and cold seasons. Generally, the incidence of plasmid was ~ 25-50% higher in bacteria isolated from site (B) compared to that recovered from site (A). Moreover, the plasmid incidence was much correlated to the origin of bacterial isolates than to the collection seasons. Further, the plasmid incidence in bacteria isolated from deep levels was higher compared to those isolated from surface in both two seasons at both two sites.

In this study, the plasmid sizes ranged from 25 to 200 Kb were considered as a large plasmid and the plasmid sizes less than 25 kb as a small plasmid. The molecular sizes of the plasmids isolated from the bacterial strains were found to be in the range of 25 to 200 Kb. The incidence of large plasmids of the total population from site (B) was greater than that of the total population from site (A). Approximately 32% of bacterial isolates from site (A) harbored large plasmids and ~ 45% harbored small plasmids and ~ 21% harbored both large and small plasmids during hot season. However, during cold season, ~ 36% of isolates harbored large plasmids and ~ 44% harbored small plasmids and ~ 19% harbored both large and small plasmids. At site (B), ~ 38% of bacterial isolates harbored large plasmids and ~ 30% harbored small plasmids, and ~ 31% harbored large and small plasmids during hot season. However, during cold season, ~ 37% of isolates harbored large plasmids and ~ 34% harbored small plasmids and 28% harbored both large and small plasmids.

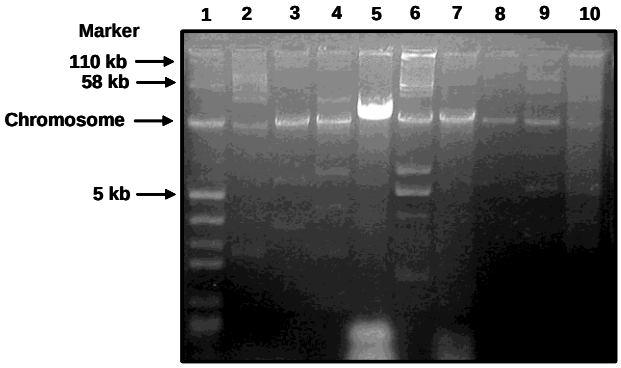

Figure 1. Visualization of plasmids in an agarose gel (0.8 %). Lane 1 represents plasmids isolated from *Shigella flexnari* which were used as a marker. Lanes 2-10 represent plasmids isolated from the multiple heavy metals and antibiotic resistance isolates from site (A) and site (B).

Antibiotic resistance bacteria

All 337 bacterial isolates which contained plasmid were screened for their resistance to antibiotic on nutrient agar by the disc diffusion method. All the isolates were screened for growth on nutrient agar supplemented with 100 µg ampicilln, 50 µg tetracycline and 10 µg/ml kanamycin. Approximately 63% and 60% of isolates from site (A) and site (B), respectively, showed resistance to ampicillin. However, ~ 53% and ~ 63% of isolates from site (A) and site (B), respectively, showed resistance to kanamycin. Moreover, ~ 33% and ~ 25% of bacterial strains from site (A) and site (B), respectively, were resistance to tetracycline.

Biodegradation

All 337 bacterial isolates containing plasmids isolated from site (A) and site (B) were screened for their ability to use/degrade phenol and two other pesticides (carbaryl and cypermethrin) as a sole source of carbon and energy. The minimal medium was supplemented with phenol, carbaryl or cypermethrin. The phenol was used as a model of aromatic ring. The results indicted that ~ 61% of the bacterial isolates from site (A) were able to assimilate 1 mM phenol as a sole source of carbon and energy however ~ 38% could not grow. Furthermore, ~ 51% of

the isolates were able to use 10 μg/mL carbaryl as a sole carbon source and energy. In addition, ~ 50% of the isolated strains were able to degrade 10 μg/mL cypermethrin.

On the other hand, ~ 64% of the bacterial isolates from site (B) were able to use 1 mM phenol and ~ 63% were able to use the 10 μg/ml carbryl as a sole source of carbon and energy. Furthermore, ~ 60% of the bacterial isolates were able to assimilate 10 μg/mL cypermethrin as a sole source of carbon and energy. One bacterial strain (*Citrobacter* sp.) isolated from site (B) was selected for the biodegradation experiment based on its high ability for carbaryl degradation designated as *Citrobacter* AA101. The growth of *Citrobacter* AA101 in MSM medium with carbaryl or glucose and in nutrient broth was followed turbdimetrically (Figure 2). The difference in turbidity (growth) is probably due to the difference in available of carbon source. The growth on carbaryl was also considerably slow and low.

Degradation of carbaryl

To detect the degradation of cabaryl, spectral scans on supernatant drawn from culture of *Citrobacter* AA101 grown in MSM medium with carbaryl as a sole source of carbon and energy was carried out. Aliquots of 1 ml were taken and prepared for spectral scans, as described in materials and methods, at zero time, 3 days and 6 days from incubation. As shown in Figure 3 that the peaks at 276 nm correspond to the absorption maxima for carbaryl. The absorbance at 276 nm was decreased with increased the time of incubation of the culture indicative the degradation of carbaryl.

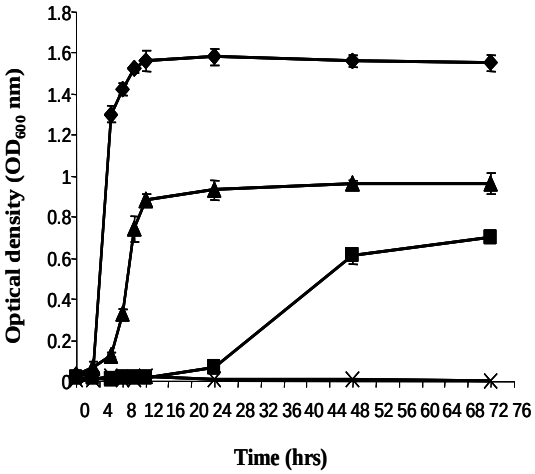

Figure 2. Growth of *Citrobacter* AA101 in minimum salt medium (MS) amended with carbaryl (■), MS plus glucose (♦), Nutrient broth (▲) and MS without any carbon source as a control (X) at 37 ^{o}C.

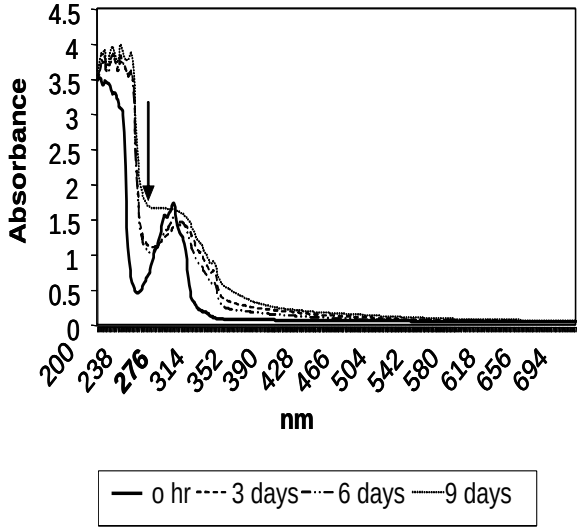

Figure 3. Spectral scans of supernatant from growing culture of *Citrobacter* AA101 in MSM medium with carbaryl at zero time, 3 days, 6 days and 9 days after incubation at 37 ^{o}C. The arrow at 276 nm indicates the absorption peak of carbaryl.

DISCUSSION

Total bacterial population in contaminated soil was more than that in non-contaminated soil. This result could be regarded as destabilization of the soil ecological balance arising from contamination. Environmental stresses brought by the contamination could be adduced for the reduction in microbial population and diversity. In our investigation, the population of bacteria in soil irrigated with canal water was ranged from 8 x 10^2 to 31 x 10^3 CFU/gm. However, in soil irrigated with wastewater, the population of bacteria was ranging from 13 x 10^2 to 45 x 10^4 CFU/gm. Previous reports have proposed high population of bacteria observed in the contaminated soil (Laukova *et al*., 2002). Previous results reported that the population of cultivable bacteria in sewage-irrigated soil was 3.36 x 10^7 CFU/gm compared with the 1.74 x 10^6 CFU/gm of the uncontaminated soil (Adesemoye *et al*., 2006). Another result concluded that the total count of heterotrophic bacteria and hydrocarbon utilizing bacteria in contaminated soil and control soil were 1.22 x 10^8 and 3.0 x 10^4 CFU/gm, respectively (Ebuehi *et al*., 2005). However, the plate viable count in control soil (without heavy metal) was in the range of 7.2 x 10^7 and 1.1 x 10^8 CFU/gm (Ahmad *et al*., 2005). There was no significant inhibition in the viable count of aerobic heterotrophs for any metals at certain concentration. A possible explanation on what happened leading to the change in population pattern is that the organisms in the wastewater and organisms autochthonous to the soil engaged in competition and other negative microbial

interactions such as antibiosis, after the wastewater was discharged into the soil. Also the CFU values were higher at the surface area than the depth in most soil samples. The bacterial density in agriculture soil irrigated with canal water ranged from 71.1 x 10^2 CFU/gm to 15.4 x 10^3 CFU/gm and from 52.3 x 10^2 CFU/gm to 10.97 x 10^3 CFU/gm in surface and depth area, respectively. However, it ranged from 12.1 x 10^3 CFU/gm to 19.9 x 10^4 CFU/gm in surface and from 10.65 x 10^3 CFU/gm to 81 x 10^3 CFU/gm in depth soils irrigated with wastewater. These results are in agreements with the data obtained by Maier *et al* (2004) concluded that the total count of cultivable bacteria isolated from surface soil samples were more than that isolated from the depth soil.

The most dominant genera of bacteria isolated from soil were *Eschershia, Shigella, Xanthomonas, Acetobacter, Citrobacter, Enterobacter, Moraxella* and *Methylococcus*. However, less diversity of Gram-positive bacteria were recovered from both sites and represented by *Bacillus, Micrococcus,* and *Staphylococcus*. The bacterial isolates identified in our study were mostly represented by Gram-negative bacteria which have been often found in wastewater-polluted soils (Trojanovska *et al*., 1997; Brim *et al*., 1999). The bacterial community of the soil irrigated with canal water was less diversity than that of soil irrigated with wastewater however the difference was not statistically difference. This conclusion could attribute to the extensive use of domestic fertilizers in the soil irrigated with canal water. The effect of water sources on the diversity of soil bacteria can not be generalized, but instead is dependent on the bacterial genera, and perhaps the species (Laukova *et al*., 2002; Malik *et al*., 2002). In our study, the diversity of *Enterobacteriaceae* increased in the soil contaminated with wastewater compared to that irrigated with Canal water. These results are in agreements with the data obtained by Meitze and Sjorgen (1983). In contrast to the effect of irrigated water source on *Enterobacteriaceae* diversity, the diversity of *Bacilli* was greatest in both sites irrigated with canal water and wastewater (Malik *et al*., 2002). However, other results have also been reported that the diversity of *Bacilli* was greatest in contaminated soil (Sagardoy and Salerno,1983).

The pollution of the environment with heavy metals has led to the appearance of heavy metal resistance microorganisms in the soil and water of industrial region. Our data indicated that high percentage of heavy metal resistance bacteria was recovered from both sites. However, the bacterial strains isolated from contaminated soil were exhibited high resistance to certain metals compared to the isolates recovered from non-contaminated soil. Other results concluded that the frequency of tolerant bacteria increases with an increase of heavy metal concentrations in contaminated soil (Angle *et al*., 1993; Kunito *et al*., 2001). The frequency of metal resistance bacteria to metal ions reflect the degree of environmental contamination with these heavy metals and may be directly related to exposure of bacteria to them (Roane and Kellogg, 1996; Hassen *et al*., 1998; Malik and Jaiswal, 2000). The MICs estimated for Cu, Cd, pb, Zn, Hg, Ni, Co, Cr, As, and Te for bacterial isolates in the contaminated soil as well as in the non-uncontaminated soil were at the levels regarded as those typical for metal-resistant species (Kunito, 1997). In addition, bacterial isolates recovered from both sites showed slightly difference in MICs for Cu, Cd, pb, Zn, Hg and Ni. Similar observation was reported by earlier researchers (Kunito *et al*., 1986; Chaudhary and Kumar, 1996). However, Bacterial isolates recovered from contaminated soil showed relatively high MICs for Co, Cr, and As in comparison with those isolated from non-uncontaminated soil (Campbell *et al*., 1995; Appanna *et al*., 1996). Bacterial isolates from soil irrigated with canal water were exhibited high MICs for tellurium (Te) in comparison with isolates isolated from contaminated soil. Previous results reported that *Enterobacteriaceace* resistance to potassium tellurite (K_2TeO_3) are found in urban sewage and were particularly prevalent in waste from a photographic processing plant (Taylor and Summers, 1979). Approximately 84% of bacterial isolates were resistant to two combined heavy metals, ~ 11% were resistant to three combined heavy metals, and ~ 1% or less was resistant to six combined heavy metals depending on the sites. These results indicated that the percentage of resistance bacterial isolates to multiple heavy metals decrease with the increasing of metal combination. Similar observations have been reported (Campbell *et al*., 1995; Appanna *et al*., 1996; Malik and Jaiswal, 2000; Aleem *et al*., 2003). Other results reported that the Majority of bacteria isolated from wastewater-treated soil showed multiple metal resistances (Malik *et al*., 2002). Moreover, previous results found that 33% of the bacterial isolates were resistant to the combination of four metals and 13.8% were resistant to six metals at a time (Aleem *et al*., 2003).

Our data indicated that heavy metals resistance has been shown to be associated with antibiotic resistance. Similar results have been reported previously (Novick and Roth, 1968; Allen *et al*., 1977). An important question addressed in this study whether exposure of soil to chemical stress such as pesticide, heavy metals and antibiotics will cause an increase in the frequency of plasmid harbored by the bacterial populations in that stressed community. Some studies have indicated that increased plasmid frequency e.g for antibiotic resistance, can occur without stress (Wickham *et al*., 1988), whereas others have found that plasmid frequency increase is a response to stress (Glassman and McNicol, 1981).

In the present study, the aforementioned parameters (pesticide, antibiotic and heavy metals) strongly suggest the presence of plasmid in the bacterial isolates. Moreover, the incidence of plasmid was ~ 25-50% higher in bacteria isolated from wastewater-irrigated soil compared to that recovered from canal-irrigated soil and approach statistical significance. Similar findings reported that ~ 91% of the total isolates from wastewater-irrigated soil were found to harbor plasmids, whereas only ~ 40% of the isolates from non-contaminated agricultural soil contained plasmids (Malik *et al*., 2002). Moreover, in the current investigation, the plasmid incidence was more

correlated to the isolates origin than to the collection sessions. Our results are supported by the hypothesis concluded that plasmid incidences are greater in polluted soil than that in bacteria from similar pristine environments (Hada and Sizemore, 1981; Ogunseitan *et al*., 1987; Campbell *et al*., 1995) and contradicting (Hermansson *et al*., 1987).

In the present investigation, ~ 28% of the isolates from non-contaminated site and ~ 43% from contaminated site were utilized 50 µL/mL carbaryl as a sole source of carbon and energy. However, ~ 25% of the bacterial isolates from both sites were able to degrade cypermethrin. *Pseudomonas* and *Rhodococcus* species which degraded carbaryl were isolated from garden soil (Larkin and Day, 1986). *Bacillus* sp. isolated from a carbaryl enrichment culture of a laterite soil and 1-naphthol and 1,4-naphthoquinone as the principal metabolites generated by this bacterium was identified (Rajagopal *et al*., 1984). Since soil microorganisms are able to metabolize an enormous range of natural and synthetic organic compounds, their adaptation to pesticides and other environmental contaminants should lead to opportunities to harness the degradative capability of microorganisms and to construct microbial isolates or consortia for cleanup of polluted environments and detoxification of hazardous wastes (Chapalmadugu and Chaudhry, 1991). Moreover, ~ 61% of the bacterial isolates recovered from site (A), and ~ 64% from site (B) utilized 1 mM phenol as a sole source of carbon and energy. These results were similar to previous findings (Kivisaar *et al*., 1989; Hinteregger *et al*., 1992; Powlowski and Shingler, 1994; Heinaru *et al*., 2000; Qureshi *et al*., 2001; Stephen *et al*., 2005).

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