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Identification And Characterization of Heavy Metal Resistant Rhizobacterial Isolates from Waste Water Soil

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Abstract

20 soil samples of agriculture soil were collected from two different sites in Gwalior, during hot and coldseasons. tensamples were from soil irrigated with canal water (site A) and ten 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 □g/mL for lead, 800 □g/mL for both Cobalt and Arsenate, 1200 □g/mL for Nickel, 1000 □g/ml for Copper and less than 600 □g/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 wasapproximately 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.

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 balancein 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 withina 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, animalsand 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 canaffect the qualitative as well as quantitative compositionof microbial communities. Several studies have foundthat 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 (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 foundto 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 bacteriain 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 irrigatedwith 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 sample

20 samples of agricultural soil were collected from different localities at Gwalior city which located at ~ 475 kilometers south from Cairo and ~ 400 kilometers north from Aswan. Twenty samples were taken from agriculturesoil 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 asupplement of essential plant nutrients. Samples were collected during the period from February 2022 to January 2023. 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 dilutionsof soils in saline solution (0.9% NaCl) on nutrient agarand incubated at 37 ºC for 48 h. The developed colonies were counted in plates and the average number of colonies per three plates was determined. The number oftotal 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 ofSystematic Bacteriology (Krieg and Holt, 1984).

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 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.

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 □g/mL Cu, ~ 41 % to 200□g/mL Co, ~ 94 % to 600 □g/mL Pb, ~ 40 % to 400 □g/mL Zn, ~ 90 % to 400 □g/mL Ni , ~ 53 % to 50 □g/mL Cd, ~ 45 % to 27 □g/mL Te, ~ 43 % to 400 □g/mL As, ~ 63 % to 200 □g/mL Cr and ~ 34 % to 20 □g/mL Hg. However, in site (B), ~ 83 % of bacterial isolates were resistant to 200 □g/mL Cu, ~ 42 % to 200 □g/mL Co, ~ 100 % to 600 □g/mL Pb, ~ 32 % to 400 □g/mL Zn, ~ 100 % to 400□g/mL Ni, ~ 27 % to 50 □g/mL Cd, ~ 37 % to 27 □g/mL Te, ~ 48 % to 400 □g/mL As, ~89 % to 400 □g/mL Cr and ~ 25 % to 20 □g/mL Hg. The highest MICs observed were 1200 □g/mL for Lead, 800 □g/mL for Cobalt and Aarsenate, 1200 □g/mL for Nickel, 1000 □g/mL for Copper and less than 600 □g/mL for other metals. These results indicated that high percentage of heavy metal resistance bacteria among theisolated strains wastewater compared to the bacterial strains isolated from soil irrigated with the canal water.

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 ourinvestigation, 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.

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