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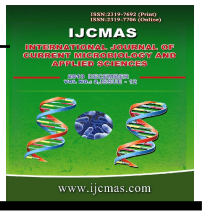

Original Research Article

Isolation and Characterization of *Bacillus* resistant to multiple heavy metals

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A B S T R A C T

Keywords
Antibiotics resistance, multiple tolerance, Heavy metal resistant bacteria.

In the present study total three heavy metal resistant *Bacillus spp* were isolated from the industrial effluents from Kanpur against Cr, Pb, Hg, Hg, Cd, Zn and Co, All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 50μg/ml to 300μg/ml. maximum microbial tolerance of *Bacillus spp* (SG-1) to Cr (280 μg/ml) and lowest to Chromium (100 μg/ml). Whileas (SG-3) maximum tolerance to Cr and Pb (200 μg/ml) and lowest to Co (100 μg/ml). And other hand all isolates showed multiple antibiotic resistant.

Introduction

The bioremediation of heavy metals using microorganisms has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application in industry. Metal accumulative bioprocess generally falls into one of two categories, bisorptive (passsive) uptake by nonliving, non growing biomass or biomass products and bioaccumulation by living cells (Macaski and Dean, 1989; Aksu and Kutsal, 1990; Huang *et al*., 1990; Volesky *et al*., 1992; Avery and Tobin, 1993; Brady and Duncan, 1994; Aksu, 1998; Doenmez and Aksu, 1999;2001). Bacterial endophytes can stimulate contaminant disappearance by the accumulation and transformation of heavy metals and some xenobiotic compounds. Several authors have investigated the role of endophytes in phytoremediation and they have found that certain plant-bacterial associations can increase bioremediation processes (Burd *et al*., 2000). Heavy metal contamination in the environment has become a serious problem due to the increase in the addition of these metals to the environment, which cannot be degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain (Igwe *et al.*, 2005). These heavy metals not only influence the microbial population by affecting their growth, morphology, biochemical

activities and ultimately resulting in decreased biomass and diversity (Roane *et al.*, 2000), but also plants and animals, but the degree of toxicity varies for different organisms. Heavy metals may decrease metabolic activity and diversity as well as affect the qualitative and quantitative structure of microbial communities (Giller *et al.*, 1998).

Some metals such as Zn, Cu, Ni and Cr are essential or beneficial micronutrients for plants, animals and microorganisms, whereas others, such as Cd, Hg and Pb have no known biological and/or physiological functions. However, all these metals could be toxic at relative low concentrations (Gadd, 1992). When exposed to moderate heavy metal concentrations, soil microorganisms were found to be very sensitive (Giller *et al*., 1998). Several studies have shown that metals adversely influence microorganisms (Shi *et al*., 2002), affecting their growth, morphology and activities (Baath *et al*., 1998; Lakzian *et al*., 2002; Khan and Scullion, 2002).

Materials and Methods

Sample collection

The sampling area was from the industrial effluents from Kanpur U.P. India, Samples was collected in sterile plastic bottles. A total of five samples were taken for the study.

Isolation and identification of heavy metal resistant bacteria

Isolation of *Bacillus* spp. Isolates

The isolation of *Bacillus* spp. from soil samples, 1g of soil sample was serially diluted in sterile distilled water, 0.1 ml of soil suspension from 10^{-1} to 10^{-6} was spreaded on the nutrient agar plate. Plates were incubated at 35^0C for 2-4 days in inverted position. (Farah *et al*., 2006).

Identification of *Bacillus* spp.

The bacterial isolates were identified by using cultural, morphological and biochemical characteristics features described in Bergey's manual of determinative bacteriology (Holt *et al*., 1994) and stored at 4°C on slants and maintained through sub-culturing. The isolates were characterized by Gram staining, motility test, Methyl Red, Voges Proskauer, Citrate, oxidase test, catalase test, H_2S production and starch hydrolysis as per the standard methods (Cappuccino and Sherman, 1992).

Heavy metal tolerance (Cervantes *et al*., 1986)

The selected bacterial strains were tested for their resistance to heavy metals by agar dilution method. Freshly prepared agar plates were amended with various soluble heavy metal salts namely Cr, Pb, Hg, Hg, Cd, Zn and Co, at various concentrations ranging from 50 to 300 μg ml-1 were inoculated with overnight grown cultures. Heavy metal tolerance was determined by the appearance of bacterial growth after incubating the plates at room temperature for 24-48h.

Determination of antibiotic sensitivity and resistance pattern

Antibiotic sensitivity and resistance of the isolated heavy metal resistant isolates were assayed according to the Kirby-Bauer disc diffusion method given by Bauer *et al*., (1996). After incubation, the organisms were classified as sensitive or resistant to an antibiotic according to the diameter of inhibition zone given in antibiotic disc table.

Table.1 Heavy metal tolerance among *Bacillus* spp. from the industrial effluents from Kanpur U.P. India

***Bacillus* Isolates**	**Cr**	**Pb**	**Hg**	**Cd**	**Zn**	**Co**
SG-1	280	200	150	175	100	140
SG -2	250	180	125	150	100	125
SG -3	200	200	180	150	125	100

Table.2 Antibiotic sensitivity and resistant activity of heavy metal resistant *Bacillus* spp.

***Bacillus* Isolates**	**Sensitive**	**Resistant**
SG-1	Amikacin, Gentamycin, Norfloxacin, Vancomycin, Ofloxacin	Methicilin, Cotrimoxazole, Cefixime, Bacitracin, Ampicillin, Amoxycillin, Cefalexin, Kanamycin, Tetracycline
SG -2	Norfloxacin, Ofloxacin, Vancomycin, Bacitracin, Amikacin,	Methicilin, Cotrimoxazole, Cefixime, Cefalexin, Ampicillin, Amoxycillin, Kanamycin, Tetracycline, Gentamycin, Chloramphenicol.
SG -3	Ceftriaxone, Ofloxacin, Amikacin,	Amoxycillin, Amikacin, Ampicillin, Cefalexin, Chloramphenicol, Kanamycin, Methicillin,

Results and Discussion

Isolation and identification of heavy metals resistant bacteria

Three heavy metal resistant *Bacillus* spp. were isolated from industrial effluents from Kanpur U.P. India, against multiple heavy metals. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 50μg/ml to 300μg/ml. All isolates showed multiple tolerances to heavy metal and were multi antibiotic resistant. Heavy Metal tolerance Test indicated highest tolerance to Chromium (280 μg/ml) by SG-1 no. isolates and lowest to Zink by SG-1 and SG-2 while as SG-3 also lowest to Cobalt (100 μg/ml).

Most of the isolates in the present study showed multiple tolerances to both heavy metals and antibiotics. It was observed that most of the metal tolerant strains (SG-1, SG-2, and SG-3) were resistant to amoxycillin, ampicillin, amikacin, bacitracin, kanamycin, methicillin, gentamycin, and tetracycline whileas Senstive to vancomyain, ofloxacin, and ceftriaxone (Table-2).

The present study showed some resemblance with the long back work of Calomiris *et al*., (1984) who found a correlation between the resistance to high level of Cu (II), Pb(II), Zn(II) and antibiotic in the bacterial species found in drinking water Vajiheh *et al.*, (2003) also studied that multiple metal resistance bacterial isolates exhibits high resistance towards a group of antibiotics.

Heavy metal resistant microorganisms play an important role in the bioremediation of heavy metal contaminated soils (Ray and Ray 2009; Rai *et al.*, 2007). Bioremediation is the use of microorganisms to break down toxic and hazardous compounds in the environment (Acquaah, 2004). It generally tilizes microbes (bacteria, fungi, yeast, and algae), although higher plants are used in some applications. The two main biological treatment processes under investigation are: the adsorption of Cr (VI) onto microbial cells (*i.e.* biosorption), and the reduction of Cr (VI) to Cr(III) by enzymatic reaction or indirectly by reducing compounds produced by micro-organisms (*i.e.* biotransformation) (Cheung and Gu, 2003; Desjardin *et al*., 2003). The biological reduction of hexavalent chromium has attracted increased interest, since this process may not only relieve the toxicity of chromium that affect living organisms, but may also aid in the precipitation of chromium at near-neutral pH (mainly as Cr $(OH)_3$) for further physical removal (Cheung and Gu., 2003).

In conclusion, our findings indicate that bacterial populations belonging to the genus *Bacillus* spp. exhibit not only adaptive response against multiple heavy metals tolerance but also they have multiple antibiotic resistant condition

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