ISOLATION AND CHARACTERIZATION OF *PSEUDOMONAS* RESISTANT TO HEAVY METALS CONTAMINANTS

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ABSTRACT

In the present study total eight heavy metal resistant *Pseudomonas sp* were isolated from sewage of industrial effluents from waste water treatment plant of Paonta Sahib H.P. India, against chromium, copper, nickel, cadmium. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 50µg/ml to 350µg/ml. All isolates showed multiple tolerances to heavy metal and were multi antibiotic resistant. Heavy Metal Tolerance Test indicated maximum microbial tolerance of *Pseudomonas sp* (Ps-6) to Copper (300 µg/ml) and lowest to Chromium (60 µg/ml).

Keywords: Antibiotics resistance, multiple tolerance, Heavy metal resistant bacteria (HMRB)

INTRODUCTION

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Copper, chromium, cadmium and nickel are known to be the most commonly heavy metals used and the more widespread contaminant s of the environment[1-3]. Traces of these heavy metals are necessary as Co-factors of enzymatic reactions, but high levels of them may cause extreme toxicity to living organisms due to inhibition of metabolic reactions. The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction reactions[4-9]. 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 (passive) uptake by nonliving, non growing biomass or biomass products and bioaccumulation by living cells[2,5,10-12]. Industrial wastes containing toxic metals were characterized by their differences rather than their similarities. These toxic metals can arise from a wide variety of industrial processes. The quality and the quantity of the wastes containing toxic heavy metals are dependent upon their industrial sources. Intrinsic bacteria, which are capable of metal accumulation, existing in soil on or near the site of contamination have adapting mechanisms to the contaminant. Naturally occurring bacteria that are capable of metal accumulation have been extensively studied since it is difficult to imagine that a single bacterium could be capable to remove all heavy metals from its polluted site[13]. Apparently, the metal, which has been introduced into the bacterial suspension by vigorous mixing, forms complexes with various ligands available (constituents which will complex heavy metal ions)[14,15]**.** Consequently, the largest amount of metals will be found as hydroxide or as a stable metal-ligand complex. Under a specific stress conditions, a relatively constant amount of metal reacts to stable and inactive complexes with active cellular components[16]**.** However, it is very important before the optimization of the bacterial growth process is to study at which pH value will be found as metal ions to study the real interaction between the free metal ions and the bacterial strain. This study aimed to isolate and characterization of *Pseudomonas sp* resistant to heavy metals contaminants from industrial effluents waste water treatment plant of Paonta Sahib H.P.

MATERIALS AND METHODS

Sample collection

The sampling area was the sewage of industrial effluents waste water treatment plant of Paonta Sahib H.P. Samples was collected in sterile plastic bottles. A total of twenty samples were taken for the study.

Isolation and identification of heavy metal resistant bacteria

For the selective isolation of heavy metals resistant bacteria, heavy metals incorporated media were used. The *Pseudomonas* isolation agar incorporated with heavy metals like $Cr6+$, $Ni2+$, $Cd2+$ and $Cu2+$ were prepared. The concentration of each heavy metal was maintained at 50 µg/ml of the medium. The wastewater sample directly streaked on (P.I.A.) media and incubated at 37°C for 24 h. After the incubation period the plates were observed for any kind of growth on the media. The isolated and distinct colonies of *Pseudomonas sp* on the selective media were sub cultured and obtain in the form of pure culture and identified on the basis of their morphology and biochemical characters.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the heavy metal resistant Pseudomonas sp grown on heavy metals incorporated media, against respective heavy metal was determined by gradually increasing the concentration of the heavy metal, 10 µg/ml each time on P

I Agar plate until the strains failed to give colonies on the plate. The starting concentration used was 50µg/ml. The culture growing on the last concentration was transferred to the higher concentration by streaking on the plate. MIC was noted when the isolates failed to grow on plates.

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 standard antibiotic disc chart.

RESULTS AND CONCLUSION

Isolation and identification of heavy metals resistant bacteria

Eight heavy metal resistant *Pseudomonas sp* were isolated from sewage of industrial effluents from waste water treatment plant of Paonta Sahib H.P. India, against chromium, copper, nickel, cadmium. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 50µg/ml to 350µg/ml. All isolates showed multiple tolerances to heavy metal and were multi antibiotic resistant. Heavy Metal Tolerance Test indicated highest tolerance to Copper (300µg/ml) by Ps-6 no isolates and lowest to Chromium by Ps-8.

Antibiotic sensitivity of heavy metals resistant isolates

All the isolates were resistant to antibiotics of which Ps-6 was found to be single antibiotic resistant while the rest of the isolates were found to be multi-antibiotic resistant (Table 2). The sewage of industrial effluents waste water treatment plant of Paonta Sahib H.P. collects all the domestic as well as industrial wastewater from Paonta region. The wastewater coming from domestic and industrial sources is the appropriate environment where the microorganisms can develop resistance to heavy metals and antibiotics. The presence of small amount of antibiotics and heavy metals in the wastewater induce the emergence of Antibiotic resistance of heavy metal resistant isolates antibiotic resistant and heavy metal resistant microorganisms. Most of the isolates in the present study showed multiple tolerances to both heavy metals and antibiotics. The microbial resistance to heavy metal is attributed to a variety of detoxifying mechanism developed by resistant microorganisms such as complexation by xopolysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another[17]. Filali et al. (1999) studied wastewater bacteria isolates *Psuedomonas aeroginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus* resistant to heavy metals and antibiotics[18]. Similarly, Sharma et al. (2000) isolated highly cadmium resistant *Klebsiella* that was found to precipitate significant amount of CdS[19].

Table 1: Resistance of bacteria to other heavy metals. (Ps= *Pseudomonas* isolates)

BACTERIA	RESISTENCE TO	MIC
Ps-1	Ni^{+2}	180 µg / ml
	Cd^{2+}	120 µg / ml
	Cu^{+2}	180 µg / ml
	Cr^{+6}	130 µg / ml
Ps-2	Ni^{+2}	125 µg / ml
	Cd^{2+}	120 µg / ml
	Cu^{+2}	180 µg / ml
	Cr^{+6}	120 µg / ml
Ps-3	Ni^{+2}	110 µg / ml
	Cd^{2+}	80 µg / ml
	Cu^{+2}	220 µg / ml
	Cr^{+6}	140 µg / ml
Ps-4	Ni^{+2}	140 µg / ml
	Cd^{2+}	90 µg / ml
	Cu^{+2}	180 µg / ml
	Cr^{+6}	110 µg / ml
Ps-5	Ni^{+2}	80 µg / ml
	Cd^{2+}	150 µg / ml
	Cu^{+2}	280 µg / ml
	Cr^{+6}	120 µg / ml
Ps-6	Ni^{+2}	200 µg / ml
	Cd^{2+}	120 µg / ml
	Cu^{+2}	300 µg / ml
	Cr^{+6}	70g µg / ml
Ps-7	Ni^{+2}	100 µg / ml
	Cd^{2+}	120 µg / ml
	Cu^{+2}	180 µg / ml
	Cr^{+6}	70 µg / ml
Ps-8	Ni^{+2}	250 µg / ml
	Cd^{2+}	210 µg / ml
	Cu^{+2}	190 µg / ml
	Cr^{+6}	60 µg / ml

The heavy metal resistant organism could be a potential agent for bioremediation of heavy metals pollution. Multiple tolerances occur only to toxic compounds that have similar mechanisms underlying their toxicity. Since heavy metals are all similar in their toxic mechanism, multiple tolerances are common phenomena among heavy metal resistant bacteria. In wastewater, there are some substances that have the potential to select for antibiotic resistance even though they are not antibiotics themselves. Heavy metals and biocides are two of them. The exposure to heavy metals or biocides results in the selection of bacterial strain also able to resist antibiotics. This happens because genes encoding heavy metals and biocides are located together with antibiotic resistance genes or alternatively because bacteria can have unspecific mechanism of resistance common to different substances including heavy metals, biocides and antibiotics[20]. It is therefore, likely that selective pressure by one such compound indirectly selects for the whole set of resistances.

Table 2: Antibiotic sensitivity and resistant activity of heavy metal resistant *Pseudomonas sp*

S. no	Bacteria	Senstive to	resitant to
1	Ps-1	Meropenem, Bacitracin, Vancomycin, Chloramphenicol, Erythromycin, Tetracycline, Amikacin and kanamycin	Ampicillin, Cotrimoxazole, Ciprofloxacin, Tetracycline
2	Ps-2	Ampicillin, Cotrimoxazole, Ciprofloxacin, Chloramphenicol, Erythromycin, Amikacin and kanamycin	Tetracycline, Meropenem, Bacitracin, Vancomycin
3	Ps-3	Ampicillin, Meropenem, Bacitracin, Vancomycin, Chloramphenicol, Erythromycin, Tetracycline and Amikacin	Cotrimoxazole, Ciprofloxacin, kanamycin
4	Ps-4	Ampicillin, Cotrimoxazole, Ciprofloxacin, Meropenem, Bacitracin, Vancomycin and kanamycin	Chloramphenicol, Erythromycin, Tetracycline, Amikacin
5	Ps-5	Cotrimoxazole, Ciprofloxacin, Meropenem, Bacitracin, Vancomycin, Chloramphenicol and Erythromycin,	Ampicillin, Tetracycline, Amikacin kanamycin
6	Ps-6	Ampicillin, Ciprofloxacin, Tetracycline, Meropenem, Bacitracin,Vancomycin, Chloramphenicol, Erythromycin, Tetracycline, Amikacin and kanamycin	Cotrimoxazole
7	Ps-7	Ampicillin, Cotrimoxazole, Ciprofloxacin, Vancomycin, Chloramphenicol, Erythromycin, Amikacin and kanamycin	Tetracycline, Meropenem, Bacitracin
8	Ps-8	Cotrimoxazole, Ciprofloxacin, Tetracycline, Meropenem, Bacitracin, Tetracycline, Amikacin and kanamycin	Ampicillin, Vancomycin, Chloramphenicol, Erythromycin

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