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Pollution

Elixir Pollution 31A (2011) 1981-1984

ISSN: 2229-712X

Metal and Antibiotic Tolerance Potentiality of *Acidithiobacillus Spp* and *Pseudomonas spp* from waste Dumps of bauxite and Magnesite mines

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ARTICLE INFO

Article history:

Received: 28 January 2011;

Received in revised form:

20 February 2011;

Accepted: 23 February 2011;

Keywords

Mine soil;

Heavy metal;

Bacterial strains;

Antibiotics;

Resistance.

ABSTRACT

The investigation was focused on the isolation of metal tolerant and antibiotic sensitive bacteria (*A. ferrooxidans* from bauxite and *P. aeruginosa* from magnesite mine) from the waste dump of mine by using selective medium. These two organisms showed maximum metal resistant potentiality for the selected heavy metals (Mn, Zn, Fe, Cr, Cu and Hg) in the range of 20 to 100 μg/ml^{-1}. The tolerance among the isolated bacteria on heavy metals were observed in order of Mn > Zn > Fe > Cr >Cu>. These organisms did not show effective tolerant to Hg. The minimal inhibitory concentration (MIC) of heavy metals (Mn, Zn, Fe, Cr, Cu and Hg) for the test bacteria were in the ranges of 50 to 200μg/ml^{-1}. The antibiotic susceptibility of these two metal tolerant bacteria were analyzed by standard antibiotics, the results showed that most (8 antibiotics) of the antibiotics are sensitive except amphicillin and co-trimoxahole. The overall results indicate that the isolated metal tolerant bacteria would be very useful for the reclamation of mine soil without any hazardous effects.

Introduction

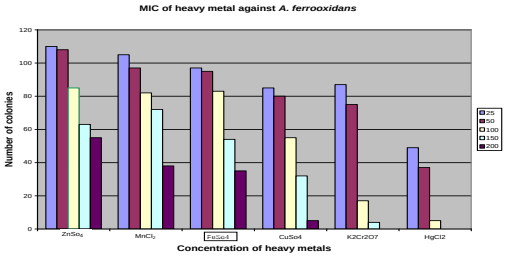
The mining industry are play an vital role in the economy of every country mean time the wastage or effluents (soil) causing the environmental pollution due to without proper disposal of waste. In India and elsewhere in the world, metal mining has been dabbed ecologically and environmentally not fully acceptable, due to unscientific exploitation of earth's resources degrade land, improper disposal mining waste lead to collapse the soil nature surface and ground water and forest cover these are could be seriously contaminated and polluted over extensive regions. It has been estimated that over 2 x 10^9t of environmentally hazardous mined and processed wastes could be generated per year due to mining activity in India (Natarajan 2009). The effluents processed soil of mining industry contains high heavy metals (such as Mn, Cu, Cr, Hg, Zn, Fe, Cd, Pb, As and Co etc.) are highly toxic to the environment by when their quantity increased in the soil. These are affecting all groups of organisms and ecosystem processes, including microbial mediated processes (Giller et al., 1998; Muller et al., 2001; Giller et al., 2009). The adverse effects of heavy metals on soil biological properties such as soil microbial biomass, soil ATP concentrations, dehydrogenase activity and N_2-fixation by rhizobia and blue green algae (Chander and Brookes, 1993; Olivera and Pampulha, 2006: Kelly et al., 1999; Lorenz et al., 1992; Chaudri et al., 2008) have been well documented so far. Several kinds of bacteria have been reported by researchers from the heavy metal containing environment (koryak and Michael, 1997). Among them, the *Acidithiobacillus spp* are characterized by their ability to oxidize elemental sulfur and other sulfur compounds (Kelly and Harrison, 1989.). *A. ferrooxidans* is able to grow by oxidation of ferrous iron or sulfidic ores; it is the most important bacterium in bioleaching (Kelly 1988; Straube et al., 2003) and evaluates the bioremediation potentiality of *P. aeruginosa* by producing surfactants on PAHs (13, 00 mg/kg) and it could tolerate to certain heavy metals (Heipieper et al., 1995). The microorganisms resistant to tolerant to metals and antibiotics appear as the result of exposure to metal contaminated environments which cause coincidental co-selection for resistance factors for antibiotics and heavy metals. Microbial resistance to antibiotics and metal ions is a potential health hazard because these traits are generally associated with transmissible plasmids. Several studies have been reported that metal tolerance and antibiotic resistance of microbes (Foster 1983; Ramteke 1997). The aim of this paper was to isolate the metal tolerant (Zn, Cu, Mn, Hg, Cr, Fe) bacteria from mining site and test the antibiotic sensitivity.

Materials and Methods

Source of soil sample and Site description

The soil samples were collected from bauxite mine (covered 469.9 km^2) (Latitude 11°816'01'39 & Longitude 78°22'33'53) at Shervarayan hill and surrounded farm/garden (considered as control), located in the northern part of Salem district (Figure 1), The soil is red, loamy and lattice and this area is made up of Archaean crystalline rock like amphibolites, leptynites, garnetiferous granites. Bauxite and magnesite are the chief mineral resources. The mean annual rainfall is 1638 mm at the upper hill and 850 mm at the foothill. The temperature ranges from 13 to 29°C on the peaks and 25 to 40°C at the foothill. The collected samples were transported to the laboratory immediately for enumerate the bacteria.

Figure 1



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Bacterial screening

The soil sample (1g) was dissolved in 10ml of double distilled water under sterile condition. Subsequently 1ml of suspension was added to 9ml of sterile distilled water to obtain desired dilutions up to 10^{-6} 100µl of two dilutions (10^{-4} & 10^{-5}) was inoculated in a nutrient agar by using standard spread and pour plate method. The plates were incubated at 37°C ±1°C for 24 hrs.

Bacterial characterization

Two suspected colonies (*Acidithiobacillus spp* and *Pseudomonas aeruginosa)* were inoculated on 9K medium (Natarajan 2009) and Centrimide agar medium (Mathiyazhagan, et al., 2011). The acidophilus were characterized by in the range of pH was 3 to 6 respectively. About100 ml of these selective medium were taken in 250 ml conical flask, the suspected cultures were inoculated on appropriate medium and the flasks were incubated at 30°C for two days on rotary shaker under 170 rpm/min. The isolated bacteria were characterized by gram staining, biochemical tests and utilization of reduced forms of sulfur (H, S, So, S, O) and metal sulfides (Ledin and Pedersen, 1996) were performed (data not shown). The results were compared with standard Bergey's manual and earlier reports.

Metal tolerant test

The isolated *A. ferrooxidans* and *P. aeruginosa* were adopted to test the metal tolerant potentiality with Zn, Mn, Cu, Cr, Hg and Fe by using the modified method of Tuhina Verma et al., (2001). The young cultures were inoculated aseptically on the nutrient agar plates supplemented individually with 6 different metals (Zn, Mn, Cu, Cr, Hg and Fe) in various range of 20 to 100 µg /ml^{-1} by using spread plate method. The inoculated plates were incubated at 30°C for 3 to 5 days. After the appropriate incubation, the colonies were counted.

MIC of heavy metals for isolated bacteria

The metal tolerant bacteria were adapted to MIC based on the method described by Tuhina Verma et al., (2001). The nutrient agar plates supplemented with different concentrations (25 to 200µg/ml^{-1}) of various heavy metals ($ZnSO_4$, $MnCl_2$, $HgCl_2$, $CuSO_4$, $K_2Cr_2O_7$ and $FeSO_4$) were inoculated aseptically with culture of *A. ferrooxidans* and *P. aeruginosa* in exponential growth phase. The plates were incubated for 36-48 hrs at 30°C and the test and sterile control plates were also maintained (Plates with culture without metals and plates without cultures and metals respectively). The minimal inhibitory concentration of heavy metal of which no colonies was observed in the plates was considered the MIC of the isolate. The isolate exhibiting growth after 3 days incubation at 30°C was considered tolerant to the metal.

Antibiotic susceptibility test

The metal tolerant *A. ferrooxidans* and *P. aeruginosa* were also adapted to test their anti bacterial resistant potential by using disc diffusion method (Tuhina Verma et al., 2001). The isolated metal tolerant *A. ferrooxidans* and *P. aeruginosa* cultures were inoculated on Muller Hinton agar by spread plate method and place the commonly available antibiotics impregnated disc (mcg/disc) on the top of agar plate (such as Gentamycin (10), chloramphenical (30), Ampicillin (10), Amoxycillin (10), Endofloxacin (10), Ciprofloxacin (10), Doxycline hydrochloride (250), Neomycin (25), co-trimazole (25) and Amikacin (25mcg) (Hi Media Chemicals, Mumbai, India). The inoculated plates were incubated at 35°C for 24 hours, after the incubation to measure the zone of inhibition. The results were classified as resistant or sensitive (Tuhina Verma et al., 2001) and antibiotic resistant index (ARI) were calculated as described by Hinton et al., (1985).

Results

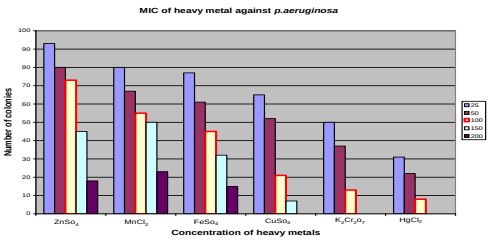
Metal tolerability

The metal tolerant bacteria viz. *A. ferrooxidans* and *P. aeruginosa* were isolated from the effluents (soil) of magnesite mines and were treated with various concentrations (20 to 100 µg/ml^{-1}) of six different heavy metals (Z_nSo_4, $MnCl_2$, $CuSo_4$, $K_2Cr_2O_7$, $FeSo_4$ and $HgCl_2$). The results showed about 75 to 26 numbers of colonies of *A. ferrooxidans* and 69 to 14 colonies of *P. aeruginosa* were observed from 20 to 100µg/ml^{-1} concentration of $MnCl_2$ (Table 1a). The *P. aeruginosa* showed high tolerability on $ZnSo_4$ than *A. ferrooxidans*, because of the more number of colonies (94 to 47) was observed in the same concentration (20 to 100 µg/ml^{-1}) of Zn (Table 1b). The average number of colonies of *A. ferrooxidans* and *P. aeruginosa* were observed on $FeSo_4$ and $CuSo_4$ containing plates (Table 1c and Table 1d) and these two bacteria were exhibited less effective tolerability on Cr and Hg at higher concentration compare to $MnCl_2$ and Z_nSo_4 (Table 1e & Table 1f).

MIC determination

The isolated metal tolerant bacteria (*A. ferrooxidans* and *P. aeruginosa*) were studied to determine the minimal inhibitory concentration (MIC) for the metals. The MIC values suggest that the resistance level against individual metal dependent on the metal tolerability of isolated bacteria. Among the two bacteria, *A. ferrooxidans* were observed effective result than *P. aeruginosa* (Fig.1).The MIC of *A. ferrooxidans* and *P. aeruginosa* were observed at 200µg/ml^{-1} of few heavy metals ($ZnSO_4$, $MnCl_2$ and $FeSO_4$) except $CuSO_4$, $K_2Cr_2O_7$ and $HgCl_2$ (Fig.2). The *P. aeruginosa* was susceptible to $CuSo_4$, $K_2Cr_2O_7$ and $HgCl_2$ (Fig.8) than *A. ferrooxidans* (at 200µg/ml^{-1} concentration).

Figure 2

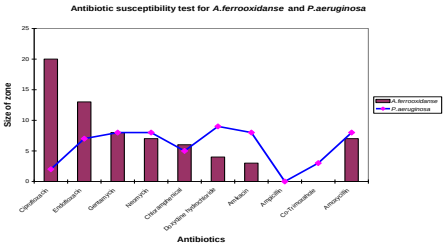


Antibiotic susceptibility

The heavy metal tolerant *A. ferrooxidans* and *P. aeruginosa* bacteria showing susceptibility to some antibiotics are presented in figure 3. The susceptibility and resistant of the bacteria was analyzed by based on the inhibition zone. The size of the zone was above 3mm means it considered to be susceptible, less than 3mm was considered as resistant (Tuhina Verma et al., 2001). The results of antibiotic susceptibility and resistant of these two bacteria was differed. The *A. ferrooxidans* was susceptible to several antibiotics (Ciprofloxacin, endofloxacin, gentamycin, neomycin, chloramphenical, doxycline hydrochloride and amikacin) and resistant to two antibiotics (ampicillin and co-trimoxahole). The *P. aeruginosa* were susceptible to doxycline hydrochloride, amikacin, gentamycin, ciprofloxacin, endofloxacin, chloramphenical, and neomycin and resistance to ampicillin, and co-trimoxahole. The *A. ferrooxidans* and *P. aeruginosa* were reported to resistant to 2 antibiotics (ampicillin and co-trimoxahole) as well as them highly susceptible to

remaining 8 antibiotics (zone ranges from3 to 20mm in *A. ferrooxidans* and in *P. aeruginosa* it was 3 to 9mm). The *A. ferrooxidans* highly sensitive to ciprofloxacin showed (20mm) highest zone of inhibition.

Figure 3



Discussion

The present study highlights the occurrence of heavy metal tolerant bacterial population in waste dump soil of mining industry. The isolates were identified as *A. ferrooxidans* and *P.aeruginosa,* these bacteria were observed tolerant to selected heavy metals (Zn, Mn, Cu, Cr, and Fe) except mercury and also show resistant to some common antibiotics. Among the two bacteria *A. ferrooxidans*, was exhibited more tolerance to heavy metal compared to *P. aeruginosa*. The association between resistance to antibiotics and heavy metals has been reported by several workers across the world (Schottel et al., 1974; Dhakephalkar and Chopade, 1994; Ramteke, 1997). The prevalence of such metal tolerant *Acidithiobacillus spp* is ecologically important, particularly if they are also antibiotic resistant, under environmental conditions of metal stress, such metal and antibiotic resistant populations will adapt faster by the spread of R-factors than by mutation and natural selection, thus, leading to a very rapid increase in their numbers (Bhattacherjee et al., 1988). We analyzed the *A. ferrooxidans* show high resistant to Zn metal than other metals. A similar observation was also made by Basu et al., (1997). In the present study, we found that the *A. ferrooxidans* and *P. aeruginosa* from magnesite mine soil were sensitive/resistant to standard antibiotics.

Conclusion

The present study was indicates that the heavy metal containing environment especially the waste soil of magnasite and bauxite mining industry have found some metal tolerant and antibiotic sensitive/resistant bacteria. The present investigation conclude that the *A. ferrooxidans* and *P aeruginosa* posses more metal resistant power (Zn, Mn, Fe, Cr, Cu) as well as antibiotic resistant potentiality against ampicillin and co-trimoxahole and more sensitive to more remaining antibiotics. Further investigation is required to determine the bioremediation potentiality of *A. ferrooxidans* and *P aeruginosa* on heavy metal containing environment.

Acknowledgements

The authors would like to express our sincere thanks to Department of Biotechnology, Periyar University, Salem district, Tamil nadu, India for providing laboratory facility for conducting this work.

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Table 1 Metal tolerant test of *A.ferrooxidans* and *P. aeruginosa* on various metals

(a) $MnCl_2$

S.No	Name of the bacteria	$MnCl_2$ µg/ml concentration and number of colonies				
		20	40	60	80	100
1	*A. ferrooxidans*	75	70	68	41	26
2	*P. aeruginosa*	69	65	55	32	14

(b) $ZnSo_4$

S.No	Name of the bacteria	$ZnSo_4$ µg/ml concentration and number of colonies				
		20	40	60	80	100
1	***A.*** *ferrooxidans*	86	82	75	61	31
2	*P. aeruginosa*	94	88	75	78	47

(c) $FeSo_4$

S.No	Name of the bacteria	$FeSo_4$ µg/ml concentration and number of colonies				
		20	40	60	80	100
1	*A.ferrooxidans*	78	67	61	58	28
2	*P. aeruginosa*	75	76	55	42	19

(d) $CuSo_4$

S.No	Name of the bacteria	$CuSo_4$ µg/ml concentration and number of colonies				
		20	40	60	80	100
1	*A.ferrooxidans*	69	55	47	45	22
2	*P. aeruginosa*	63	58	38	27	15

(e) $K_2Cr_2O_7$

S.No	Name of the bacteria	$K_2Cr_2O_7$ µg/ml concentration and number of colonies				
		20	40	60	80	100
1	*A.ferrooxidans*	81	67	40	27	11
2	*P. aeruginosa*	56	38	22	18	10