International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 05 Issue: 08 | Aug 2018 www.irjet.net p-ISSN: 2395-0072

Potential risks associated with bacterial strains isolated from heavy metal rich soil of a landfill area

Sayantan De[1], Shaunak Ghosh[2]

[1,2]Department of Biotechnology, Heritage Institute of Technology, Kolkata

---

Abstract - Dumping of Industrial wastes in the water and landfill areas causes heavy metal contamination which is adversely affecting the environment. Among all the heavy metals Arsenic, Mercury and Nickel are among the few of most abundantly found toxic metals in contaminated areas, posing serious threat to the ecosystem. Our experimental study was based on these three metals. The result of change in the ecosystem with the increasing concentration of such toxic elements in the nature has tremendous effect on the bacterial population as well. In order to adapt with the new environment few bacterial species are developing tolerance towards high concentration of these heavy metals which may develop as new pathogens creating new form of diseases. On the other hand, they can have great utility in the process of bioremediation and waste management in future. Our aim in this project was to isolate newer strains of such bacterial species which can survive high concentration of heavy metals in the soil. We also have tested their antibiotic resistance property to evaluate the potential threat they may pose on human lives, if they prove to be pathogenic in nature. A landfill area in Jalpaiguri district, West Bengal was chosen to collect the soil sample for our experiment. Then heavy metal (Ni^{2+} , Hg^{2+}, and As^{3+}) tolerant bacterial colonies were isolated and further characterized both morphologically and biochemically.

Key Words: Landfills, Heavy metals, Heavy metal tolerant, Antibiotic-Resistant

1. INTRODUCTION

Metals whose atomic density is more than 5 $g/cm^3$are defined as heavy metals [7]. Heavy metals cannot be degraded or destroyed and hence they are treated as stable, persistent contaminants of the environment [8]. Some heavy metals are essential for living but are required only in very small quantity as micro nutrients and hence are known as "trace elements". If present in slightly higher concentration than what is needed they can cause severe harm to the living organisms. Many agricultural and industrial and even domestic practices are responsible for release of such heavy metals in the environment creating pollution of land and water [9]. Mining power stations and the application of pesticides, fertilizer in the agricultural fields and sewage sludge are few of the sources of heavy metal contamination in the soil [10]. Apart from plants and animals, microbes are also very much affected my heavy metal contaminations. Indigenous microbial populations face severe challenges due to such contaminations as these can alter microbial activities and even nitrogen fixation properties of soil bacteria. These causes damage to the cell membrane and can alter enzymes specificity thereby hampering cellular functions. Even at certain instances they can also alter or damage the structure of the DNA [11]. But having the advantage of shorter life cycle, the microbes can adapt very fast to the changing environment.

Heavy metal contamination is significantly higher in sewage and dumping areas (landfills)[1]. Such landfill areas are also populated with wide varieties of unknown microorganisms. Very few works have been done to evaluate bacterial diversity of such landfill areas. Microbial populations in heavy metal polluted environments contain bacteria which have been adapted to toxic concentrations of different heavy metals like Ni, As, Hg and become metal resistant. Those microorganisms developed wide mechanisms for survival in the presence of highly toxic heavy metals, and acquired certain genetic properties [3]. They have evolved mechanism to either eliminate or detoxify such metals within their cells. Many such bacterial strains have already been isolated having the property to tolerate high concentration of heavy metals. It has been found in many experiments that many of those bacteria which are resistant to the effects of high concentrations of heavy metals in their ecosystem are often resistant to several antibiotics.

In this study, our aim was to isolate several strains of bacterium from landfill damp soil and toidentify the particular strains which have the ability to tolerate different concentration of heavy metals along with evaluation of their antibiotic resistance ability. The relationship between heavy metal tolerance and antibiotic resistance properties of isolated bacteria with its plasmid content has been previously correlated in many studies. In recent years, the disposal of industrial wastes containing heavy metals,mainly in the nearby water bodies, has resulted in an increase in population of resistant bacteria. The results are indispensably more common in industrial hubs. Discharge of wastewater containing heavy metals into rivers causes accumulation in soil particles (in the form of clay) and transfer of these to far away places [12]. The results of some studies show that, in landfill areas, the concentration of heavy metals in less settable particles such as clay, can travel to distance places. Some researchers have indicated that the rate of metal (As, Ni, Hg...) exhibition concentration in West Bengal, India exceeds the quality guideline for the concentration of heavy metals in sediments. Since soil is one of the most important environments for microbes and is

easily exposed to many pollutants, evaluating the effects of pollutants on the microbial population is very valuable.

2. Materialsand Methods

2.1 Sample collection

Soil sample were collected from a landfill area of North Bengal ,India.

2.2 Tests performed

pH test of the soil sample was performed then one gram of soil sample was taken and serial dilutions were carried out in sterile water. Dilutions viz., 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were prepared and are used for the isolation of microorganisms[5]. In order to isolate above microorganisms the nutrient agar growth media has been used.Isolation of heavy metal resistant bacteria done from different heavy metal added in nutrient medium in different concentrations,1(M)$NiCl_2$,1(M) $NaAsO_2$ and 0.5(M) $HgCl_2$. Three isolated heavy metal colonies were taken and further biochemical characterizations were performed [4]. Gram staining was performed to know the morphological properties of the

Isolated heavy metal tolerant strains were grown in nutrient media containing Ampicillin and Chloramphenicol in order to check its antibiotic resistant properties.

3. Results

The pH of soil sample was also measured and found to be 8.04.

Table-1. Summary of Gram's Staining for Heavy Metal resistant organisms

Heavy Metals	Strain A	Strain B	Strain C
$NiCl_2$	(-) ve	(+) ve	(+) ve
$HgCl_2$	(+) ve	(+) ve	(+) ve
$NaAsO_2$	(+) ve	(+) ve	(-) ve

Table-2. Summary of Biochemical Tests for Heavy Metals Resistant Organisms.

Heavy Metals	Biochemical Tests	Strain A	Strain B	Strain C
$NiCl_2$	Urease Test	(-) ve	(-) ve	(-) ve
	Catalase Test	(+) ve	(+) ve	(+) ve
	Starch Hydrolysis	(+) ve	(-) ve	(-) ve
	Gelatin Hydrolysis	(-) ve	(-) ve	(-) ve
	Indole Test	(-) ve	(+) ve	(+) ve
	MR Test	(+) ve	(+) ve	(+) ve
	VP Test	(-) ve	(-) ve	(-) ve
	Citrate Test	(+) ve	(+) ve	(+) ve
$HgCl_2$	Urease Test	(-) ve	(-) ve	(-) ve
	Catalase Test	(+) ve	(+) ve	(+) ve
	Starch Hydrolysis	(-) ve	(-) ve	(+) ve
	Gelatin Hydrolysis	(-) ve	(+) ve	(-) ve
	Indole Test	(+) ve	(-) ve	(-) ve
	MR Test	(-) ve	(-) ve	(+) ve
	VP Test	(-) ve	(-) ve	(-) ve
	Citrate Test	(+) ve	(+) ve	(+) ve
$NaAsO_2$	Urease Test	(-) ve	(-) ve	(-) ve
	Catalase Test	(+) ve	(+) ve	(+) ve
	Starch Hydrolysis	(-) ve	(-) ve	(+) ve
	Gelatin Hydrolysis	(-) ve	(+) ve	(-) ve
	Indole Test	(-) ve	(-) ve	(-) ve
	MR Test	(-) ve	(-) ve	(+) ve
	VP Test	(-) ve	(-) ve	(-) ve
	Citrate Test	(+) ve	(-) ve	(+) ve

Table-3. Summary of determination of both antibiotic and heavy metal resistant bacteria

Antibiotic	**Strain A**	**Strain B**	**Strain C**
Ampicillin	(+) ve	(+) ve	(-) ve
Chloramphenicol	(+) ve	(-) ve	(-) ve

Gram staining of the samples revealed that most of the colonies were Gram positive bacillus in shape and two strains were Gram negative. These different strains were chosen for further work.

4. Discussion

This study was conducted from a soil sample taken from landfill in Jalpaiguri district of West Bengal, as landfill areas are found to have high concentration of Arsenic, Nickel and

Mercury. Bacterial colonies dwelling in such areas are there by expected to have heavy metal tolerance property. The three strains isolated from the soil sample are also tolerant to the above mentioned three heavy metals as they could grow on an agar media supplied with different concentrations of those metals. The strains were separately grown on agar media with each of the heavy metals added one at a time. From the result obtained, it is evident that strain A tolerate Arsenic and Mercury, strain C can tolerate Nickel and Mercury whereas strain B has the property to tolerate all the three heavy metals. Investigations lead to conclusions of the presence of the heavy metal resistant organisms in the same soil sample which can be responsible for greater health hazards as they also have developed antibiotic resistance property (apart from the strain C) [6]. This gives an example of how human development and civilization affects even the genetic makeup of living organisms, especially microbes, creating new, more tolerant species which may someday challenge the mankind with new, untreatable diseases. On the other hand, the heavy metal resistant organisms can be used as detector of pollutants present in the environment. Bioremediation of contaminated waste water and sewage treatment can be done by isolated heavy metal strains.

REFERENCES

[1] . Cheng, S. (2003) Heavy metal pollution in China origin, pattern and control Environmental Sciences and Pollution Research 10, 192-198.

[2].Mindlin S., Kholodii G., Gorlenko Z., Minakhina S., Minakhin L., Kalyaeva E., Kopteva A., Petrova M., Yurieva O., Nikiforov V. Mercury resistance transposons of Gram-negative environmental bacteria and their classification. Res. Microbiol. 2001;152(9):811–822.

[3].Siddiquee S, Rovina K, Al Azad S, Naher L, Suryani S, et al. (2015) Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: A review. J Microbial BiochemTechnol 7: 384-393.

[4]. Chien, C., Y. Kuo, C. Chen, C. Hung, C. Yeh and W. Yeh, 2008. Microbial diversity of soil bacteria in agricultural field contaminated with heavy metals. Journal of Environmental Sciences, 20: 359-363.

[5]. Shanmugam et al. Isolation and characterization of microorganisms from rhizosphere soil and root samples of Meliadubia trees, Species, 2013, 4(10), 6-9.

[6]. K.E. Giller, E. Witter, S.P. McGrath, Toxicity of heavy metals to microorganisms and microbial process in agricultural soils: a review, Soil Biol. Biochem. 30 (1998) 1389–1414.

[7]. Järup L 2003, "Hazards of heavy metal contamination", British Medical Bulletin, vol. 68, no. 1, pp. 167–182, doi:10.1093/bmb/ldg032.

[8]. Kwiatkowska, B., Elferink, A.G.O., Molenaar, E., Soons, A.H.A. International Organizations and the Law of the Sea: Documentary Yearbook 1996. Springer Netherlands. Edition: 2. Page: 441. ISBN - 9789041110046. https://books.google.co.in/books?id=6GOVS_0Zm6oC.

[9]. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metals Toxicity and the Environment. *EXS*. 2012; 101:133-164. DOI:10.1007/978-3-7643-8340-4_6.

[10]. Raymond A. Wuana and Felix E. Okieimen, "Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation," ISRN Ecology, vol. 2011, Article ID 402647, 20 pages, 2011. https://doi.org/10.5402/2011/402647.

[11]. Khatun M, Bera P, Metra D, Mandal A and Samanta, A. Estimation of heavy metals tolerance and antibiotic susceptibility of Bacillus cereus isolated from municipeal solid waste. International J. of Pharma. Bio. Sciences. 2012; 3: 819-829.

[12]. Kermanshahi, R. K., Ghazifard, A. and Tavakoli, A. (2007), Identification of bacteria resistant to heavy metals in the soils of Isfahan province. Iranian Journal of Science & Technology, Transaction A. 31, pp 7-16.