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Frequency of Bacterial Chromium Resistance in Different Oil Contaminated Water and Soil Samples, Tiruchirappalli City

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Abstract

In this study, the water and soil sample were collected from the four different oil contaminated regions of Tiruchirappalli city during premonsoon and monsoon season 2015. In which, a total of sixty (60) bacterial strains were isolated and challenged against chromium metal (Potassium chromate) solutions with four different concentrations (10 mM, 50 mM, 100 mM and 250 mM) for metal resistant studies by plate diffusion and tube dilution methods. A growth rate between 90-100% was observed for 86.5 % of the bacterial populations at 10 mM of Cr, whereas no population was growing at a growth rate of 0–80 % with 10 mM of Cr. At 50 mM of Cr, 53.5 % of the populations showed a growth rate of 81-90 % while 10 % of the population showed a growth rate of 0-80 %. A growth rate between 51-60 % was observed for 1.5 % of the bacterial populations at 100 mM of Cr, whereas no population was growing at a growth rate of 0–50 % with 100 mM of Cr. At 250 mM of Cr, 33.5 % (20 isolates) of the populations showed a growth rate of 71-80 % whereas 38.5 % of the populations showed a growth rate of 61-70 % in 250 mM concentrations. Interestingly, 1.5 % of the strains were survive in the 250 mM concentration and most of the strains were resistant to minimum level concentration (10 mM) of chromium metal. Our results show that Tiruchirappalli oil contaminated regions has a significant proportion of heavy metals and metal resistant bacteria, and these bacteria constitute a potential risk for public health.

Keywords: Oil contamination, Metal resistant bacteria, Copper, Chromium, Tiruchirappalli.

Introduction

Pollutants in both terrestrial and aquatic environments are derived from both point and non-point sources. Waste disposal operations intentionally release materials to water shed via direct dumping and pipeline discharges, which constitute point sources of pollution. The dumping of municipal sewage sludge and industrial wastes (acid-iron waste, alkali chemicals, pharmaceuticals and etc.), and the discharge of domestic/industrial effluents from outfalls are the primary point source categories. Non-point sources of pollutants also originate from human/ animal activities with accidental releases (domestic sewage, oil spills, urban runoff, septic tank leakage, groundwater transport, erosion, contaminated soils, and atmospheric deposition), marine mining, and the operation of vessels. Pollution of the natural environment by heavy metals is a worldwide problem as these metals are indestructible and have toxic effects on living organisms when they exceed a certain concentration limit (MacFarlane and Burchett, 2000).

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Heavy metals in environment mostly come from lithogenic and anthropogenic sources. Discharge of urban and industrial waste water, combustion of fossil fuels, mining and smelting operations, processing and manufacturing industries, waste disposal including dumping, etc., are primary anthropogenic sources of pollution (Yu et al., 2001). Through the natural process of biomagnifications, minute quantities of metals become part of the various food chains and concentrations become elevated to levels which can prove to be toxic to both human and other living organisms (Kishe and Machiwa, 2003). The heavy metals like Cd, Cu, Cr, Hg and Pb, may exhibit extreme toxicity even at low levels under certain conditions, thus necessitating regular monitoring of sensitive aquatic and terrestrial environments (Peerzada et al., 1990). Microorganisms which survive in the extreme environment can develop its resistance behavior to adopt themselves to extreme environments including toxic heavy metals. It has been suggested that the metal resistance may not be a casual phenomenon and bacterial resistance against toxic metals appears to be directly related to the presence of these elements as environmental pollutants (Silva and Hofer, 1993). Biosorption/resistance of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals (Hussein et al., 2004). The direct impact of heavy metal pollution on microbial ecosystem includes the alterations in the physiology, diversity and abundance of microorganisms, which indirectly affect the biogeochemical cycles and aquatic productivity (Haferburg and Kothe, 2007; Bong et al., 2010). The aim of this study was to determine the level of chromium resistant bacteria from the oil contaminated regions of Tiruchirappalli city.

Materials and methods

Study area

The Tiruchirappalli city is one of the largest municipality and big urban agglomeration in the Tamil Nadu state. Tiruchirappalli being sited almost at the centre of the state. Many industrial and densely residential areas have recently been developed in all part of the city and around 1.5 million people are living in this city. According to the 2011 Indian census, Tiruchirappalli had a population of 847,387, 9.4% of whom were under the age of six, living in 214,529 families within the municipal corporation limits (https://en.wikipedia.org/wiki/Tiruchirappalli; Vinothini and Ravikumar, 2016).

Sampling

The water and soil sample were collected from the four different oil contaminated regions of Tiruchirappalli city during premonsoon and monsoon season 2015. The 500 mL of oil contaminated water samples were collected with a 1000 mL sterile container in each locations. The oil contaminated soil samples were collected by sterile spatula and stored in sterile plastic bags and stored in ice box at 4 °C (Kumarasamy et al., 2009). The samples were transported into laboratory and processed within 12 hrs (Vignesh et al., 2012; 2013; 2015). The sampling sites are Ponmalai Railway Shed (PRS), Senthaneerpuram Oil Shed (SOS), Chatram Bus Stand (CHB) and Central Bus Stand (CLB). The sampling sites were choose based on the oil pollution. In which the sampling sites were divided in to two categories such as oil shed (PRS and SOS) and oil waste mixing with sewage (CHB and CLB).

Heterotrophic bacterial studies

A total of sixty (60) bacterial strains were isolated from water and soil samples of the sampling sites. The serial dilution and pure culture techniques were used for isolation of bacterial strains and were used as test cultures (Muthukumar et al., 2015). The fifteen strains were isolated from the each location and were identified by the specific biochemical tests (Rapid Microbial Limit Test kits used) (Vignesh et al., 2014; Muthukumar et al., 2015).

Metal resistant studies

The test isolates were challenged against chromium metal (Potassium chromate) solutions with four different concentrations (10 mM, 50 mM, 100 mM and 250 mM) for metal resistant studies by plate diffusion and tube dilution methods. In plate diffusion assay, the 500 µL of chromium metal solution (four different concentration) was added to a central well (1 cm in diameter and 4 mm in depth) of nutrient agar plate separately and to allow it for metal diffusion at one day. In each metal concentration plate, eight bacterial isolates were inoculated in each plate by the radial streaking method. In tube dilution method (Minimal inhibitory concentration method), the appropriate volume of metal solution and 200 µL of standard culture (10^8 CFU/mL) were added into nutrient broth medium and make up into 10 mL with sterile nutrient broth. The test plates and tubes incubated at 37±1 °C for 48 h (Hassen et al., 1998). All the trials were performed in triplicate. In plate diffusion method, the percentage of bacterial tolerance was calculated in terms of the ratio: length of growth in mm *vs* length of the total inoculated streak while in the tube dilution method, direct visible reading was carried.

Result and discussion

Microorganisms undergo selection pressures in the presence of toxic compounds and develop resistance (Hideomi et al., 1977). The most common resistance is to metal and antibiotics, which can be a result of bio-essentiality or of abuse of the metal, and/or antibiotics. Enumeration of this resistant group from different geographical locations (Mudryk et al., 2000) has shown that these groups are ubiquitous. Residual effects of the most of these heavy metals on aquatic biota are long lasting, as they can be non available due to complex formation with organic matter. Thus they are not easily eliminated from these ecosystems (Forstner and Wittmann, 1983). The presence of the organisms that possess specific mechanisms of resistance to heavy metals increases destruction or transformation of toxic substances in the natural environment (Souza et al., 2006). For example, at millimolar concentrations, Zn ions bind with the cell membrane of bacteria and interfere with cell division (Nies, 1999) in spite of being a micronutrient. Brynhildsen et al. (1988) stated that the pathogens from Arabian marine waters were highly resistant to the heavy metals.

In this study, sixty bacterial strains were isolated from the oil contaminated regions and were challenged against different concentrations of chromium metal solutions. Interestingly, only few of the isolates to higher concentration of Cr (K_2CrO_4) exhibited resistance. The present study, the bacterial isolates were highly sensitive to chromium metal depend on metal concentrations. Concentration depended manner play an important role in the metal resistant studies. Interestingly, more than 85 % of the strains were resistant to minimum level (10 mM) of metal concentration while 1.5 % of the strain were resistant to chromium metal at high concentration (250 mM) (Table 1). This study indicated that the increasing concentration of the metal solution affects the bacterial growth at considerable level. When the concentration of metal solution increased the microbial growth could be gradually reduced. Toxic metal pollution exerts a pressure on microbial community leading to the emergence of resistant strains with apparent reduction in the extracellular enzyme activity of that particular ecosystem (Souza et al., 2006).

A growth rate between 90-100% was observed for 86.5 % of the bacterial populations at 10 mM of Cr, whereas no population was growing at a growth rate of 0–80 % with 10 mM of Cr. At 50 mM of Cr, 53.5 % of the populations showed a growth rate of 81-90 % while 10 % of the population showed a growth rate of 0-80 %. In 100 mM of Cr, 33.5 % of the populations showed growth rates between 81-90%, whereas 51.5 % of the populations were observed with a 71-80 % growth rate. At 100 mM Cr, 13.5 % of the populations showed 61-70 % growth rate. A growth rate between 51-60 % was observed for 1.5 % of the bacterial populations at 100 mM of Cr, whereas no population was growing at a growth rate of 0–50 % with 100 mM of Cr. At 250 mM of Cr, 33.5 % (20 isolates) of the populations showed a growth rate of 71-80 % whereas 38.5 % of the populations showed a growth rate of 61-70 % in 250 mM concentrations. A growth rate between 51-60 % was observed for 18 % of the bacterial populations at 250 mM of Cr, whereas 10 % of population was growing at a growth rate of 0–50 % with 250 mM of Cr. This study indicated that most of the bacterial strains were resistant to chromium metal and which can be easily survived in the oil contaminated regions. Based on this result, we may conclude that these strains could be used to biosorption studies as bio-sorbents. On the other hand, routine monitoring is needed to avoid pollutions in these regions.

Table I. Percentage of isolated chromium resistance strains from oil contaminated regions of Tiruchirappalli, Tamil Nadu

Percentage of growth	Oil contaminated regions - Bacterial isolates (*n* = 60)							
	Chromium (Cr) metal solution							
	10 mM		50 mM		100 mM		250 mM	
	N	%	N	%	N	%	N	%
0-10 percentage of growth	-	-	-	-	-	-	-	-
11-20 percentage of growth	-	-	-	-	-	-	-	-
21-30 percentage of growth	-	-	-	-	-	-	-	-
31-40 percentage of growth	-	-	-	-	-	-	-	-
41-50 percentage of growth	-	-	-	-	-	-	06	10
51-60 percentage of growth	-	-	-	-	01	1.5	11	18
61-70 percentage of growth	-	-	-	-	08	13.5	23	38.5
71-80 percentage of growth	-	-	06	10	31	51.5	20	33.5
81-90 percentage of growth	08	13.5	32	53.5	20	33.5	-	-
91-100 percentage of growth	52	86.5	22	36.5	-	-	-	-
	Minimal inhibitory concentration (MIC) of chromium – Bacterial strains – (*n* = 60)							
	10 mM		50 mM		100 mM		250 mM	
Resistant strains	60		51		39		01	

N / n – Numbers; mM – Milli Molar; % - Percentage

Conclusion

This study concluded that most of the bacterial isolates were resistant to chromium metal solution with different concentrations and it leads to affect the microbial growth. An other hand, this situation may provide the select pressure on the bacterial community and it make the strains as resistant. In this study, most of the strains were resistant to minimal (10 mM) concentrations whereas very few strains could be survive in the (resistant) high (250 mM) concentration. This situation may therefore pose a threat to natural environments and to human health. To minimize this threat, more detailed monitoring studies should be carried out. Heavy metal resistance of bacteria should be examined in detail to determine whether or not it is due to chromosomal or plasmid origin.

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