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Research Article

ISOLATION OF METAL RESISTANT BACTERIA FROM MUNICIPAL SOLID WASTE DUMPSITE, MADURAI

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ABSTRACT

Hevy metals are the major pollutant in Municipal Solid Waste (MSW). Naturally microorganism grow well on the polluted site, breakdown complex and toxic substances into simple forms which they can utilize in their metabolic processes for growth. Aim of the study is isolation ofeffective metal resistant bacteria from municipal solid waste.The municipal solid waste sample was collected from Avaniapuram dumpsite Madurai. Twenty twodifferent bacterial strains were isolated named as AM01-AM22 andsevenmetal resistant bacterial strains named asAMHM01-AMHM07 from solid waste sample.The quality of solid waste analysed by various physico-chemical parameter.Bacterial isolates that are able to grow on nutrient agar incorporated with heavy metals viz., Zn2+, Cu4+ , Pb4+ and Cr3+ were isolated from municipal solid waste sample and were identified as *Pseudomonas aerogenosa , Pseudomonas fluorescens, Pseudomonasputida, Bacillus cereus , Bacillus subtilis ,Staphyllococusaureus and Bacillus thuringiensis.* These bacteria have the ability to grow in minimum concentration of four metals but their metal tolorence level is vary. *Pseudomonas aerogenosa ,Staphyllococusaureus* grow on 450 µg/ml of Zn2+. *Pseudomonas putida, Bacillus thuringiensis*observed on 250 µg/ml of Cu4+, *Staphyllococusaureus* observed on 200 µg/ml of Pb4+ , and *Pseudomonas putida , Staphyllococusaureus , Bacillus thuringiensis* observed on 150 µg/ml of Cr3+.The results conclude that heavy metal resistant organism could be a potential agent for bioremediation of heavy metals pollution.

Keywords: Heavy metal resistant bacteria; Municipal solid waste; Minimum inhibitory concentration; Pollutant and Bioremediation.

INTRODUCTION

Rapid urbanization and population growth increased the level of municipal solid waste. Municipal solid waste (MSW) is highly polluted with heavy metals from industries, house hold and agricultural activities. Heavy metals constitute one of the major pollutant groups that arekept under surveillance in leachate from landfills for municipalsolid waste .Landfills are the most widely used disposal method across the world. According to LovleenGupta *et al*., 2014, MSW landfill is not a safe method of disposal, which are biochemically active and toxic substances are gradually leached and released into the surrounding environment.Due to migration process of leachate, soils have been contaminated with heavymetals such as lead, copper, zinc, manganese, chromium and cadmium and theseheavy metals in soils lead to serious problems as they cannot bebiodegraded (Hong *et al*., 2002). The main sources of heavy metals in the municipal solid waste are, pharmaceuticals, certain detergents, personal care products, fluorescent tubes, garden pesticides, photographic chemicals,waste oil, batteries,wood treated with dangerous substances, electronicwaste, electricalequipments, and paint etc., generated at the household (Slack *et al* ,.2005).The concentrations of metals in the leachate, which can varywidely from the microgram to the milligram per litreconcentration (Christensen *et al*., 2001), and also some significant fluctuation appear on heavy metal levels due to seasonal variation and environmental condition.(Malyuba Abu Daabes*et al*., 2013). Metals are transported to aerosol by two ways one is the transport of the fine material enriched with metals from MSW dumpsite. The second is the emission of heavymetals from the uncontrolled selfignition and the incineration residue including metals in suspected to the aerosol and transported by winds (Mohamed and Elsayed 2007).Ashok Kumar *et al*., 2010 reported thatmetals play an intrinsic role in the living organisms. Some metals likeCa, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn are essential for metabolism and regulation of osmotic pressure. Trase amount of metals serve as micronutrients. While many other metals like Ag, Al, Cd, Au, Pb, and Hg have no biological role and they are nonessential. They enter our body via food, drinking water and air. When it exceeds the level can lead to poisoning.Achiba *et al*.,2009 and Mohamed rashad*et al.,*2011 concluded in their report application of MSWC for five successive years increased and accumulation of heavy metals in A horizon of soil.To reduce hazardous pollution from the environment bioremediation is the best choise. Bioremediation is the process by the use of microorganisms to reduce or remove the pollutant from the contaminated site. According to GarimaAwasthi*et al*., 2015 two methods of bioremediation technologies is present. One is the Intrinsic which makes use of microorganism occring naturally to degrade contaminants without engineered interventions at the site. The another method involves engineered bioremediation is to alter environmental conditions for enhancing microorganisms activity to remove heavymetals. This study was aimed to isolateand identify heavy metal resistant bacteria from municipal solid waste dumping site near Madurai.And compare theirefficiency level of metal resistance.

MATERIALS AND METHODS

Site Description:

Madurai is second largest city in Tamilnadu, with total population of 14, 62,420 (2011 census).The current solid waste generations from the city is about 593mt/day. This waste is disposed by open dumping at the Vellakal, Madurai (Figure.1). Total area of this site is about 110 acre.The City is situated on 9^0 55' N latitude and 78^0 7' E longitude. The climate of Madurai town is hot and dry and temperature ranges between a maximum and minimum of 420C and 210C respectively. April through June is usually the peak summer season. Rainfall is irregular and intermittent, with an annual average of approximately 850 mm.

Figure 1: Madurai municipal solid waste dumping site

Sample collection:

Solid waste sample were collected from the surface of the open dumping area (1 m depth) at MSW dump site on April 2015.100 gof samples were collected from four different location and mixed properly. Which are placed in sterile plastic bags and labeled.Then taken to the laboratory for pre-treatment and analysis.

Physicochemical analysis of municipal solid waste:

After transportation, in the laboratory the bulk solid samples were spread on trays and were air dried at ambient conditions for two weeks. Thesamples were then grounded by mortar and pestle, sieved

through a 2 mm mesh, and oven-dried at 50◦C for about 48 hours and were stored at room temperature before analysis. Chemical characteristics of samples wereanalysed for the following properties: pH(pH meter);Moiture (drying at 105^0 C constant weight by gravimetric method);Total organic carbon(cold oxidation with potassium dichromate WalkeyandBlack method);Organic matter(ashing); Total Phosphorous (tri acid mixture with aqua digestion);Nitrate nitrogen(Brucinesulphate method);Potassium and Sodium (Flame photometer method);Sulphate (Using Spectrophotometer); and Calcium And Chloride(titration method)

Heavy metal analysis:

Samples (1.00 ± 0.001g each) were placed into 100 ml beakers separately, to which 15 ml of tri-acid mixture (70% high purity HNO3, 65% HClO4and 70% H2SO4 in 5:1:1 ratio) was added. The mixture was then digested at 80◦C till the solution became transparent (Allen *et al*.,1986).

Isolation and idendification of bacteria from municipal solid waste:

10 g of MSW sample was suspended in 90 ml sterie saline solution (8g NaCl in 1000 ml distilled water) for two hours, under shaken (150 rpm). The aqueous layer containing mixed microbial population.Theaqueous layer serially dilutedto 10^{-6} with salinesolution.Then,0.1mlof diluted suspension was plased on nutrient agar plates. These plates were incubated at 30^0C for 48-72 h.

Isolation and identification of heavy metalresistant bacteria:

Above the same procedure followed for isolation of metal resistant bacteria. After serial dilution 0.1mlof diluted suspension was plased on nutrient agar plates amended with 50 µg/ml of Zn2+, Cr3+, Pb4+ and Cu4+ .These plates were incubated at 30^0C for 48-72 h. The most frequent strains of the bacteria isolated and stored onto nutrient agar at 4^0C.

Preparation of Heavy metals concentration:

Standard solutions were prepared by dissolving the selected metals in distilled water. 1000 mg l^{-1} of each metal were prepared with deionized water. Solutions of varing concentrations (50,100,150,200,250,300,350,400 & 450µg/ml) were prepared by diluting the stock solution with deionized water. These solutions subsequently were sterilized at 121^0C for 15 min (Karakagh*et al*.,2012).

Determination of Minimum InhibitoryConcentration (MIC) for metals:

The Minimum Inhibitory concentration (MIC) is idendified as the lowest concentration of metal that inhibits the visible growth of microorganisms.The MIC of Zn2+,Cu4+, Pb4+ andCr3+ resistant bacterias were determined by the nutrient agar dilution method (Aleem*et al*.,2003). The metals were used to prepare 1000 mg ml^{-1}stock solutions in sterile deionized water. Preparation of various concentration (50,100,150,200,250,300,350,400 & 450µg/ml) of individual metal from this stock solution used for the

inoculation of isolated bacteria individually into plates.The bacteria were incubated at 30^0C for 72 h.The lowest concentration of metals that completely prevented the growth of each bacterium were considered as MIC.

RESULT AND DISCUSSION

Physico chemical characteristics of municipal solid waste:

Bacterial growth depends upon various physiochemical conditions such as media, pH, temperature, carbon source, potassium, nitrogen and Phosphorous etc. Bacteria can grow in a wide range of moisture level (Zaved*et al*., 2008). According to Carboo and Fobil 2005 moiture content is one of the most important parameter in determining the burning characteristics of a material.Table 1 shows the physic-chemical characteristics of the sample.pH can influence the solubilityof metals. The pH was 8.5 slightly alkaline nature and moisture was 52% which enhance bacterial growth.Nitrate nitrogen was analysed and expressed in mg/l (1.562).The other parameter were measured and expressed inpercentage of total organic carbon,organic matter,total phosphorous, potassium, chloride,sodium, sulphate,calcium were (18.254, 26.489, 4.65, 1.2, 3.456, 0.954, 21.45, 15.52) respectively.Four metals were selected and analysed in solid waste sample such as Zinc, Copper, Lead and Chromium The concentration of these metals were 260.52,180.35,68.25&120.12 mg/kg respectively.The solid waste characteristics are not constant itsdebends on the composition of waste, age of landfill and season.

Isolation of bacteria fom municipal solid waste:

Totally 22 different bacteria were isolated from Municipal solid waste dumping site, Avaniyapuram, Madurai and named as AM01 – AM22.The isolates were observed in nutrient agar (NA) plate. Isolated bacteria werepurified and idendifiedbaced on morphological and biochemical features following Bergey's Manual of Determnative Bacteriology (Bergy& Holt1994). Based on the morphological and biochemical characteristics theidendified bacteria were listed on table 2.

Isolation of heavy metal resistant bacteria:

Heavy metal-resistant microorganisms are thought to naturally occur in metal-contaminated environment (Klimek 2012). According to Margesin and Schinner2012microorganisms in a stressed environment especially in presence of heavy metals is dependent to development of tolerance mechanisms.Microorganism play important role in the cycling of toxic metals in the biosphere and the toxic mechanism of all heavy metals is similar,multiple tolerance are common phenomena among heavymetal resistant bacteria (Alboghobeish*et al*., 2014).Totally 7 different bacteria were isolated from Municipal solid waste dumping site,Avaniyapuram, Madurai and named as AMHM01, AMHM02, AMHM03, AMHM04, AMHM05, AMHM06 and AMHM07. The isolates were observed in nutrient agar (NA) incorporated with heavy metals viz., Zn^{2+}, Cu^{4+} , Pb^{4+} and Cr^{3+} which were resistant to fixed concentration (50µg/ml) of each of

heavy metal. The idendified bacteria were listed on table 3.

Characteristics of resistant isolates:

The bacteria that could tolerate 50µg/ml of Zn2+, Cu4+, Pb4+ and Cr3+ were selected, purified and idendifiedbaced on morphological and biochemical features following Bergey's Manual of Determnative Bacteriology (Bergy& Holt1994). Table 4Shows morphological and biochemical characteristics of the idendified bacteria.Based on the morphological and biochemical characteristics the isolates are *Pseudomonas aerogenosa*, *Pseudomonas fluorescens*, *Pseudomonasputida*, *Bacillus cereus*, *Bacillus subtilis, Staphyllococusaureus* and *Bacillus thuringiensis*. These bacteria were selected for determination of MIC.

Minium Inhibitory Concentration:

For MIC determination these isolates were streaked onto the NA medium containing metal salts and then incubated at 37°C for 24-48 hrs. The concentration of metal where there was no growth is observed as the MIC for that strain.The metal resistance of isolates showed on table 5 and figure 2. The MIC of Zn2+ for AMHM01, AMHM02, AMHM03, AMHM04, AMHM05, AMHM06 and AMHM07, isolates were 400, 100, 150, 400, 300, 450 & 350 µg/ml respectively. Likewise the MIC of Cu4+ 200,150,250,200,150,150&250µg/ml, Pb4+150,100,100,150,100,200&150 µg/ml and Cr3+50,100,150,50,100,150 &150µg/ml respectively.From this results all the isolated bacteria have the ability to grow in minimum concentration of four metals but their metal tolorence level is vary. *Pseudomonas aerogenos, Staphyllococusaureus*grow on 450µg/ml ofZn2+. *Pseudomonas putida, Bacillus thuringiensis*observed on 250 µg/ml of Cu4+, *Staphyllococusaureus* observed on 200 µg/mlof Pb4+ ,and *Pseudomonas putida, Staphyllococusaureus, Bacillus thuringiensis* observed on 150 µg/ml of Cr3+ . Table 3 & Figure 2 Shows the resistance of isolated bacteria on metals.After successful growth of metal resistant bacteria, the pure culture were subcultured in nutrientagar slants ,incubated at 37^0C to achieve vigorous growth and then preserved in 20% glycerol vials at -80^0C.(Williams and Cross 1971)

CONCLUTION

From the results it can be concluded that there are several different heavy metal resistant bacteria present in the contaminated environment, which are efficient and useful for bioremediation. Seven different heavy metal resistant bacteria isolated from the dumping site. All the isolates have multiple tolerance of 50 µg/ml Zn 2+,Cu4+,Pb4+,and Cr3+. *Staphyllococusaureus* and *Pseudomonas aerogenosa*are effective in maximum Zn resistance than other bacteria. Similarly *Pseudomonas putida*and *Bacillus thuringiensis* have maximum efficiency on Cu and Cr resistance.*Staphyllococusaureus* have highest resistance on Pb than other bacteria .These idendified bacterial strains can be valuable for the bioremediation of heavy metal polluted environment. Before the field trial isolatesshould be screen for pathogenicity and confirm their environmental friendliness.

S.No	Parameter	Concentration
1	pH	8.5
2	Moiture %	52
3	Total Organic Carbon%	18.254
4	Organic matter %	26.489
5	Total Phosphorous %	4.65
6	Nitrate nitrogen mg/l	1.562
7	Potassium %	1.2
8	Chloride %	3.456
9	Sodium %	0.954
10	Sulphate %	21.45
11	Calcium %	15.52
12	Zinc mg/kg	260.52
13	Copper mg/kg	180.35
14	Lead mg/kg	68.25
15	Chromium mg/kg	120.12

Table 1: Physicochemical analysis of municipal solid waste

Strain No	**Idendified bacteria**
AM01	*Escherichia coli*
AM02	*Klebsiellapneumonia*
AM03	*Proteus vulgaris*
AM04	*Pseudomonas aeruginosa*
AM05	*Streptococcus pyogenes*
AM06	*Serratiamarcescens*
AM07	*Bacillus cereus*
AM08	*Enterococcus faecalis*
AM09	*Shigellaflexneri*
AM10	*Bacillus megaterium*
AM11	*Pseudomonas putida*
AM12	*Staphylococcus aureus*
AM13	*Salmonella enteritidis,*
AM14	*Bacillus subtilis*
AM15	*Streptococcus salivarius*
AM16	*Pseudomonas fluorescens*
AM17	*Streptococcus faecalis*
AM18	*Mycobacterium avium*
AM19	*Staphylococcus epidermidis*
AM20	*Streptococcusfaecium*
AM21	*Bacillus thuringiensis*
AM22	*Streptococcus bovis.*

Table 2: Isolation of bacteria from municipal solid waste dumpsite

S.No	Isolates	Zn	Cu	Pb	Cr
1	*Pseudomonas aerogenosa*	+	+	+	+
2	*Pseudomonas fluorescens*	+	+	+	+
3	*Pseudomonasputida*	+	+	+	+
4	*Basilluscereus*	+	+	+	+
5	*Bacillus subtilis*	+	+	+	+
6	*Staphyllococusaureus*	+	+	+	+
7	*Bacillus thuringiensis*	+	+	+	+

Table 3: Isolation of bacteria in different metals

+ Present, - Absent

S.No	**Morphological & biochemical characteristics**	**AMHM01**	**AMHM02**	**AMHM03**	**AMHM04**	**AMHM05**	**AMHM06**	**AMHM07**
1	Gram staining	-	-	-	+	+	+	+
2	Motility	Motile	Motile	Motile	Motile	Motile	Non motile	Non motile
3	Shape	Rod	Rod	Rod	Rod	Rod	Cocci	Cocci
4	FA/OA*	OA	OA	OA	FA	OA	FA	FA

5	Catalase	+	+	+	+	+	+	+
7	Oxidase	+	+	+	+	-	-	-
8	Urease	+	+	+	-	-	+	+
9	Gelatinase	+	+	+	+	+	+	+
10	H_2S production	-	-	-	-	-	-	-
11	Nitrate reduction	-	-	-	+	+	+	+
12	Indole production	-	-	-	-	-	-	-
13	Methyl red test	-	-	-				
14	Vogusprosk aur	-	-	-	+	+	+	+
15	Citrate utilization	+	+	+	+	+	+	+
16	Glucose	-	-	-	+	+	+	+
17	Lactose	-	-	-	-	-	+	+
18	Sucrose	-	-	-	-	-	+	+
19	Isolates	*Pseudomonas aerogenosa*	*Pseudomonas fluorescens*	*Pseudomonasputida*	*Bacillus cereus*	*Bacillus subtilis*	*Staphyllococusaureus*	*Bacillus thuringiensis*

Table 4: Morphological and biochemical characteristics of isolates

* FA- facultative anaerobe / OA- obligate aerobe,+ Positive,- Negative

S.NO	STRAIN NAME	METALS	MICVALUEµg/ml
1	*Pseudomonas aerogenosa*	Zn Cu Pb Cr	450 200 150 50
2	*Pseudomonas fluorescens*	Zn Cu Pb Cr	300 150 100 100
3	*Pseudomonasputida*	Zn Cu Pb Cr	350 250 100 150
4	*Basilluscereus*	Zn Cu Pb Cr	400 200 150 50
5	*Bacillus subtilis*	Zn Cu Pb Cr	300 150 100 100
6	*Staphyllococusaureus*	Zn Cu Pb Cr	450 150 200 150

7	*Bacillus thuringiensis*	Zn Cu Pb Cr	350 250 150 150

Table 5: Resistance of isolates to different heavy metals

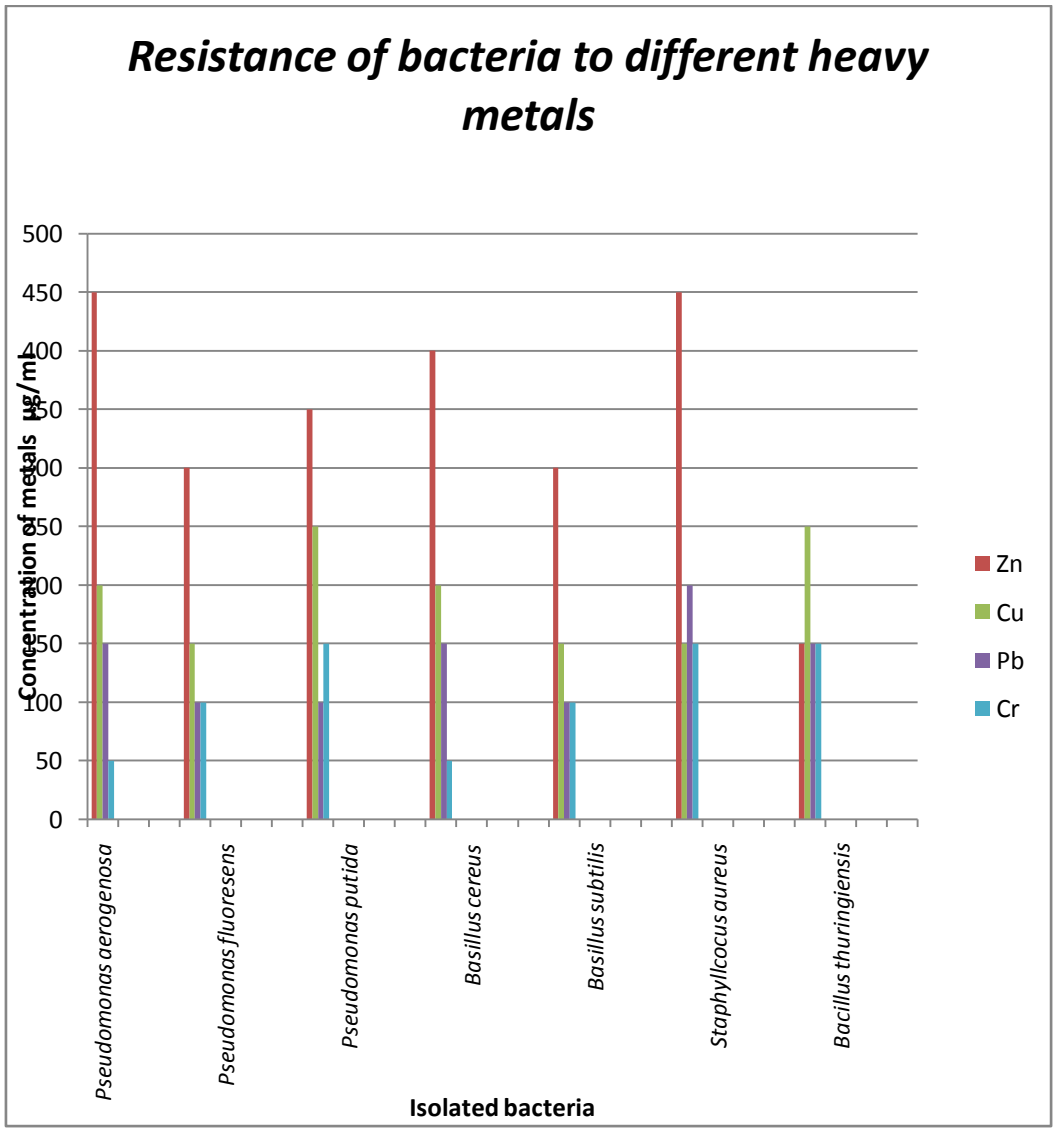

Figure 2: Resistance of isolated bacteria to different heavy metals

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