International Biodeterioration & Biodegradation 119 (2017) 467–475

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Characterization of induced metal responses of bacteria isolates from active non-sanitary landfill in Malaysia

B. Jayanthi [a, *], C.U. Emenike [a, b], S.H. Auta [a], P. Agamuthu [a, b], S.H. Fauziah [a, b]

[a] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Centre for Research in Waste Management, Institute of Research Management & Monitoring, University of Malaya, 50603 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form
27 October 2016
Accepted 30 October 2016
Available online 10 November 2016

Keywords:
Bacteria
Heavy metals
Landfills
Leachate contaminated soil
Metals resistance

ABSTRACT

Microorganisms have specific genetic mechanisms towards their resistance to heavy metals, and may exhibit tolerance by immobilizing metal on cell surfaces or transforming them into less toxic forms. Unfortunately, selecting microbes that are metal-specific is a problem in remediation processes, and require identification of a process/strategy for evaluating metal interaction and bioreduction potential of microbial species before utilization for biodegradation/reduction in polluted soils. The study aimed to express the metal tolerance and interaction within the microbial abundance in an active non-sanitary landfill soil in Malaysia, as a developmental strategy for selecting bacterial species important for future remediation of metal-polluted soil. The characterized soil exposed the contamination level from heavy metals at the selected active non-sanitary landfill. Further assessment on the microbial community identified and typed the bacterial diversity in the contaminated area. Exposure to varying metal solutions showed the sensitivity of the bacteria species. Microbiological media infused with Pb, Al and Mn solutions demonstrated absolute heavy growth for all the six microbes studied at metal concentrations of 5 −20 ppm. Comparison between the microbes indicates that *Burkholderia vietnaminesis* expressed higher metal resistance. In general all the isolated microbes demonstrated the ability to tolerate and resist metals at different concentrations. Bacterial isolates, mainly the gram-positives are metal-specific and may act as potential agent for remediation of heavy metal in contaminated sites.

1. Introduction

Whereas many sources of heavy metal contamination of soil abound, leachate pollution is one of such which is gradual but persistent, and harbours many environmental pollutants including heavy metals. Landfilling is one of the sources of heavy metals pollution due to leachate production (Agamuthu et al., 2014). The anthropogenic activities have led to their wide distribution in the environment and negatively impact human health in particular, and the ecosystem in general. The inevitable waste generation pattern, especially in the developing countries, often leads to the generation of high volume of leachate. Characterization of leachate, especially from municipal solid waste (MSW) landfills, has shown that it contains different groups of pollutants such as organics: alkenes, aromatic hydrocarbons, acids, esters, alcohols, hydroxybenzene, amides, and others, as well as ammonia nitrogen and high load of heavy metals (Emenike et al., 2013; Fauziah et al., 2013; Kjeldsen et al., 2002). Without proper collection system, raw leachate from landfills will laterally seep into soil compartments to cause soil contamination (Emenike et al., 2016). Areas near landfills have a greater possibility of groundwater contamination because of the leachate originating from the nearby site. Such contamination of groundwater resource poses a substantial risk to local resource users and to the natural environment. Heavy metal poisonousness depends on several conditions which includes level of pollutants, route of exposure, and chemical species. Health risks of leachate contamination due to groundwater contamination includes skin irritation, nausea, vomiting, and headache, while chronic exposure can lead to anemia, kidney damage, prostate cancer, lung cancer, memory loss, coma, headaches and depression. Arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to

* Corresponding author.
E-mail addresses: jayne_barath@yahoo.com, jayne.barath@siswa.um.edu.my (B. Jayanthi).

http://dx.doi.org/10.1016/j.ibiod.2016.10.053
0964-8305/

the U.S. Environmental Protection Agency (USEPA), and the International Agency for Research on Cancer. Therefore, the inevitable task faced by the society is identifying ways to prevent metal pollution in order to conserve the environment. Similarly, a more significant interest is to recover already polluted sites for the associated socio-economic benefits. Usually, the dynamic shift towards global sustainability is steering remedial or recovery activities towards the use of microbes. Hence, the use of microorganisms for cost-effective restoration of environment cannot be over emphasized. Microbes play an important role in the environmental fate of toxic metals (Polciniczak et al., 2013; Babu et al., 2013) by range of mechanisms for conversion between soluble and insoluble forms. Since heavy metals are increasingly found in microbial habitats due to natural and environmental processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (Lucious et al., 2013; Tiwary and Dubey, 2016). Microbes are capable of removing, concentrating and recovering metals through bioaccumulation or adsorption. Bacterial species found in leachate impacted environment or bioreactor influence degradation and removal of pollutants (Zhang et al., 2016), however, most studies on microbial interaction with leachate are limited to organic matters, xenobiotic organic chemicals, ammonia and few others (Gojski et al., 2012; Brkanac et al., 2014; Tigini et al., 2014) with less on heavy metals. The accumulation of heavy metals by microbes basically depends on the concentration and availability of heavy metals and is a complex process which is controlled by multiple factors, such as type of metal, the nature of the medium and microbial species. This research evaluated the difference in microbial species resident in non-sanitary landfill soil in relation to the heavy metals tolerance of the bacterial species. Bioremediation of metal pollution have received significant improvement with the use of biosorbents, especially when dead and living microorganisms are used (Wang and Chen, 2006; Gupta et al., 2010), but adsorption is often limited to metal ions from solution (Fosso-Kankeu et al., 2014) and there is need to explore microbial community for more bacterial species with the ability to concentrate metal ions. Therefore, the study aimed to express the metal tolerance and interaction within the microbial abundance in an active non-sanitary landfill soil of Peninsular Malaysia, as a developmental strategy for selecting bacterial species important for the future remediation of metal-polluted soil. Hence, its discrete objectives included diversity identification for polluted soil of active landfill and growth response to metal exposure.

2. Materials and method

2.1. Soil and leachate samplings and characterizations

Bukit Beruntung landfill site was selected for this study based on status and grade. Hence, soil samples were excavated at 30 cm depth from Bukit Beruntung landfill (BBL) (3° 32.14′N; 101° 25.80′E) in accordance to 2014 ASTME − 1197 standard guidelines for conducting terrestrial soil-core microcosm test (Emenike et al., 2012). The samples collected were analyzed for pH using multiprobe meter (YSI Professional Plus, USA), while the elemental concentrations of metals in the soil were evaluated based on the USEPA 3050B and USEPA 3052B protocol. Similarly, the raw leachate samples were collected from the environment and analyzed for similar parameters as with the soil samples. All assessments were duly replicated.

2.2. Bacteria isolation and identification

Bacteria species were isolated by mixing 1 g of soil sample with 10 ml of normal saline water (0.9% NaCl) as stock. The mixture was shaken vigorously (2 h at 180 rpm) using Lab-line 3521 orbit shaker and subjected to 20 times serial dilution. Dilutions (0.1 ml) were dispensed on freshly prepared nutrient agar under aseptic condition (Emenike et al., 2016). The plates (replicated) were incubated at 37 °C for 24 h. Developed colonies were further sub-cultured to ensure purity before identification. Subsequently, Biolog GEN III Microplate protocol was used to test the isolated microbes according to Bochner (1989a), (1989b). This involved the use of specific inoculation fluid (IF-A catalog no. 72401) to prepare suspension of the target cells. A multi-channel automated pipette was used to dispense 100 μL of the suspension prepared from inoculation fluid into each of the wells in a microplate (Catalog no.1030). The wells contain 71 carbon source utilization assays (columns 1–9) and 23 chemical sensitivity assays (columns 10–12), hence the isolates were identified at the species levels based on the “phenotypic fingerprint” of the microorganisms provided by the test panel. OmniLog reader was used to identify the bacteria species contained in the microbial identification systems software.

2.3. Heavy metal resistivity test

Bacteria isolated from the method discussed above were aseptically re-grown by inoculating each into discrete test tubes containing 5 ml of nutrient broth each at 37 °C for 18–24 h. Each inoculum was then transferred to the test tubes containing 4.5 ml of normal saline for standardization to obtain 0.1 ABS (absorbance)/0.5 McFarland at 860 nm. Final inocula required for the heavy metal sensitivity assessment were obtained by dispensing 0.1 ml of the resultant standard into corresponding test tubes containing 9.9 ml of normal saline for each test organism; hence approximate cell density of 5×10^5 CFU/ml was achieved. Therefore, the metal tolerance for each bacterial isolate was determined by agar-well diffusion method. The standard suspension of each organism (5×10^5 CFU/ml) was used to seed each sterile plate that contains 20 ml of nutrient agar. Pre-diffusion was allowed before cork borer was used to make 6 mm diameter well (Aweng et al., 2011) on the seeded plates. Four concentrations (5, 10, 15 and 20 ppm) of each metal were prepared. Table 1 shows list of metals used in this study. 70 μl of each concentration of the metals was dispensed into corresponding wells. Hence, the each plate accommodated four concentrations of designated heavy metal, and was allowed to stand 1 h for pre-diffusion. Plates were then incubated at 37 °C for 24 h. The minimum inhibitory concentrations (MIC) for the organisms were determined which is the lowest concentration at which no visible growth was observed. Diameters of the corresponding clear zones that characterized the concentrations of the heavy metals that showed no visible growth were measured to determine the inhibition zone diameter (IZD) (Jayanthi et al., 2016).

Table 1
Sources of utilized metal ions.

No	Heavy metals	Salts	Product
1	Lead (Pb)	$PbCl_2$	Merck
2	Manganese (Mn)	$MnSO_4$	Friendemann Schmidt
3	Iron (Fe)	$FeSO_4.7H_2O$	HumbG Chemicals
4	Mercury (Hg)	$HgSO_4$	Bendosen
5	Zinc (Zn)	$ZnSO_4.7H_2O$	AnalaR
6	Copper (Cu)	$CuSO_4$	Bendosen
7	Cadmium (Cd)	$CdCl_2$	Friendemann Schmidt
8	Nickel (Ni)	$NiCl_2.6H_2O$	Bendosen
9	Chromium (Cr)	$K_2Cr_2O_7$	HumbG Chemicals
10	Aluminium (Al)	$Al_2(SO_4).16H_2O$	Systerm