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Tolerance of TBT-resistant Bacteria Isolates to Methylmercury

[1]O.A. Adelaja and [2]H.E. Keenan
[1]Department of Chemistry, Federal University of Technology, P.M.B. 704, Akure, Nigeria
[2]Department of Civil Engineering, University of Strathclyde, Glasgow United Kingdom

Corresponding Author: O.A. Adelaja, Department of Chemistry, Federal University of Technology, P.M.B. 704, Akure, Nigeria

ABSTRACT

Four strains of selected TBT-resistant bacteria were tested for growth in the presence of methylmercury. Results indicate their potential of detoxification not only of TBT but also were resistant to MeHg. The EC_{50} of these four is bacterial isolates; *Pseudomonas fluorescens, Enterobacter cloacae*, *Citrobacter braakii* and *Alcaligenes faecalis* were 0.32, 0.39, 0.34 and 0.35 μM MeHg, respectively. *Enterobacter cloacae* has been shown in this study to exhibit high resistance to the toxic effects of MeHg as it is previous reported to show similar high resistance capability to TBT. These bacteria species were also examined for their biodegradability and it has found that they shows capability of degrading MeHg even in the absence of primary nutrient-glycerol, suggesting that these microorganism can utilise the carbon-source in the pollutant in order to mineralise the organic compound. This study has successively proven for the first time that these four selected TBT-resistant bacteria species were both efficient MeHg resistant and degrader. *Enterobacter cloacae* is the most preferable as highly effective resistant TBT and MeHg degrader and thus recommended for future use.

Key words: Methylmercury, tolerance, biodegradation, EC_{50}, bacterial isolates

INTRODUCTION

Methylmercury (MeHg) has been known to be a highly toxic compound which poses significant risks on both human and aquatic organisms. Owing to its high toxicity, persistence and the ease of their bioaccumulation in trophic chains, methylmercury in the environment have been of major concern for some years. Known in mythology for its fleet-footedness, mercury-a primary source of MeHg production-swiftly spreads all over the globe from its natural and anthropogenic sources. They can be released in aquatic media as pesticides, or as side products of catalytic processes in industry but can also be produced from mineral mercury through bacterial activity in sediments, as reported by Jensen and Jernelov (1969). The main point source of mercury contamination in marine environment is through the loading of the atmospheric inorganic mercury. Anthropogenic activities further added to the increasing level of total mercury in aquatic environment. Methylation and demethylation processes are the major biotic processes that occur in lakes and marine sediments and the chemical equilibrium of these two is significantly influenced by environmental factors such as sulphate and sulphide concentrations, pH and sediment organic matter level (Choi *et al.*, 1994; Okoronkwo and Olasehinde, 2007). Emissions from anthropogenic activities account for the increase in the total mercury levels in the atmosphere. MeHg enters the food chain through lower organism like the phytoplankton and others and biomagnified along this trophic level especially in larger aquatic organism.

However, consumption of fish and sea foods are the major route by which humans are exposed to MeHg contamination (Choi and Ceeh, 1998). In 1952, one of the worst anthropogenically-orchestrated disaster recorded in human history occurred at Minamata Bay in Japan where industrial waste containing mercury compounds frequently dumped into nearby aquatic bodies led to the poisoning of several thousands of people (Kojima and Fujita, 1973; Greenwood, 2006). The growing number of incidents with huge loss of human life and destruction to aquatic ecosystem prompted regulatory attention globally and is mainly administered by the United Nation for Environment Programme, UNEP (Weiss, 1995; Gray, 2002; UNEP, 2003). In recent years, there have been various global agreements and treaties on mercury emission cuts and this reflects global cooperation and commitment towards the abatement of anthropogenic mercury emissions from scientific and policy development standpoints. Several authors have successfully demonstrated robust studies on the assessment of the MeHg exposure to humans, wildlife and aquatic organism and its complex interaction with the environment (Arnot and Gobas, 2003; Boudou and Ribeyre, 1997; Cabana *et al.*, 1994; Clarkson and Magos, 2006). However, fewer works have been carried out in investigating extensively, the use of bacteria in detoxification of MeHg especially by bacterial strains previously without MeHg exposure (Mergler *et al.*, 2007; Hedayati, 2012).

For the past decade, numerous studies have propounded many methods for MeHg detoxification. More importantly, degradation by various types of bacteria have shown higher efficiency and this paves a major pathway for sustainable remediation solutions (Czuba *et al.*, 1987; Bending and Rodriguez-Cruz, 2007; Gupta and Ali, 2004; Marvin-DiPasquale and Oremland, 1998). However, it is so obvious that typical contaminated sediment would contain mixed pollutants. Therefore, a need arise for a microorganism that can effectively remediate such environment. Rather, bacteria species capable of degrading a particular toxicant is generally believed among several reports to be isolated from a sediments or surface waters contaminated with the toxicant (Spangler *et al.*, 1973; Wuertz *et al.*, 1991; Sakultanitimetha *et al.*, 2009). From previous studies, most researchers only examined the degradability efficiency of bacteria species against a single pollutant like MeHg (Kawai *et al.*, 1998; Bernat and Dlugonski, 2002; Spangler *et al.*, 1973; Wuertz *et al.*, 1991; Sakultanitimetha *et al.*, 2009). Clearly, though some authors suggested that bacteria can exhibit a multi-resistant mechanisms to detoxify various organic pollutant and heavy metals (Mergler *et al.*, 2007; De *et al.*, 2003, 2008; Khoramabadi *et al.*, 2008), there have been no reports (to our knowledge) recorded where TBT-resistant bacteria were equally investigated for MeHg tolerance. Organometals (like MeHg) can stall a variety of energy-linked reactions in bacteria; these include growth, solute transport and biosynthesis of macromolecules (Wuertz *et al.*, 1991). Therefore, this makes remediation effort ineffective as the additive effect of the variety of these organic pollutants adversely affects the microbial activity and hence lowers degradation efficiency.

Spangler *et al.* (1973) examined microcosm taken from MeHg-contaminated sediments for the capability in demethylating MeHg at certain concentration range. They observed that 30 isolated bacteria from the microcosm were tolerant at the range of 0.5-10 μg mL^{-1}. However, it can be deduced that excessive levels of MeHg can adversely impact bacterial growth. Decrease in bacterial population observed is known by counting the colony forming units after bacteria exposure to MeHg in the incubator for seven days. The chronic exposure to a toxicant can increase tolerance to that particular toxicant as long exposure increase the bacteria memory response (Blanck and Dahl, 1996; Bending and Rodriguez-Cruz, 2007; Mortazavi *et al.*, 2005). Bacteria isolated from

different sediment can shows tolerance to another toxicant in another environment. However, very low tolerance level is observed in contrast to the sediment/site it is isolated from. De *et al.* (2003) suggested that Hg-resistant bacteria were capable of growth at far higher concentration (i.e., 50 ppm Hg) than previously reported. It can be inferred that bacteria can be exhibit resistance not only to Hg but also other heavy metals such as Cd, Pb, Phenols and other xenobiotics. However, De *et al.* (2003) did not examine these microorganisms for MeHg resistance even at higher concentration. Hence, it could be presumed that bacterial isolates may exhibit multi-resistant to different deleterious organic pollutants, for example, like TBT and MeHg which are highly toxic and commonly found in aquatic environment.

Organomercurial-resistant bacteria were first isolated from MeHg-contaminated marine sediment by Spangler *et al.* (1973). Spangler *et al.* (1973) isolated bacterial species that are capable of degrading MeHg with the evidence of methane and Hg production as end products of the degradation process. These microorganisms degrade MeHg by volatilising Hg out from their cells. *Pseudomonas* sp., *Enterobacter* sp. and *Citrobacter* sp. have been isolated and found to be highly resistance to MeHg (De *et al.*, 2003; Spangler *et al.*, 1973; Mirzaei *et al.*, 2008). Most of these species mentioned earlier are gram negative and are said to have certain resistant genes which attribute for their defensive mechanism. Bacteria isolated from such sediment have been reported to have develop a surprising array of resistance mechanisms based on clustering genes in a single operon referred as "*mer operon*".Ubiquity of *mer operon* genes with respect to geographical location, environment and species allows resistant strains of bacteria and other microorganisms to thrive in presence of ionic or organic mercury compounds generally toxic to non-resistant bacteria and other forms of life (Rasmussen *et al.*, 2008). The *mer operon* that confers mercury resistance to bacteria is widely distributed in mercury-resistant bacterial populations (Osborn *et al.*, 1997; Mahmod, 2001; Barkay *et al.*, 2003; Rezaee *et al.*, 2008) which is fairly highly conserved.

Some bacterial strains, *Alcaligenes faecalis*, *Enterobacter cloacae* and *pseudomonas* sp. have been identified to be MeHg resistant at about 2.5 mg L^{-1} CH_3HgCl (Shariat *et al.*, 1979). Bacterial resistance to MeHg is determined by plasmids, which in many instances also encode resistance to other heavy metals and organometallic compounds (De *et al.*, 2003). Single bacteria strain can be resistant to many organometals. A bacteria strain, *Enterobacter cloacae,* isolated from sediment contaminated with organotin compounds has been found to also possess the capability of degrading both TBT and MeHg though at different concentration relatively lower than the former. However, previous studies revealed that the four bacteria isolates examined in this study have been known to be highly resistant to TBT and MeHg especially when they are isolated from their corresponding contaminated sediments (Shariat *et al.*, 1979; Spangler *et al.*, 1973; Wuertz *et al.*, 1991; Sakultanitimetha *et al.*, 2009).

Interestingly, this study will for the first time attempts to examine the resistance of TBT-resistant bacteria to MeHg. Anticipated positive outcome of this research study will enable us make a reasonable suggestion for the use of this kind of bacteria species for remediating sediments contaminated with two or more organometals. By and large, it is hoped that a deeper insight of scientific role in the defining environmental problems provide a lasting solution for concrete problems associated to mercury contamination.

MATERIALS AND METHODS

Chemicals: All chemicals were used without additional purification. Methylmercury chloride (96% purity) and Methanol (98% purity) were obtained from Aldrich (Steinheim, Germany). All solvents

were HPLC grade obtained from Merck (Darmstadt, Germany). All reagents used were of analytical grade. All bacteriological nutrients and agars were obtained from Oxoid (Basingstoke, UK). All other chemicals used were obtained from BDH (Poole, UK).

Sample preparation: Nutrient media and solutions: The nutrient media was prepared by adding 1.0 g of K_2HPO_4, 1.0 g of KH_2PO_4, 1.0 g of $(NH_4)_2SO_4$, 0.4 g of $MgCl_2$, 0.125 g of yeast extract and 1.0 mL of glycerol in 1 L of nanopure water and subsequently autoclaved for 3 h at 55°C while the media used for the screening experiment was made of the composition as mentioned above but the amount of the glycerol was gradually reduced till zero (Sakultanitimetha *et al.*, 2009). The media was adjusted to pH 6.8 (by adding required volume of 2 N NaOH) before been autoclaved at similar operating conditions. About 17.5 g of the Plate Count Agar (PCA) was dissolved in 1 L of nano pure water; the mixture was shaken vigorous to ensure complete dissolution and thereafter sterilized in an autoclave. The sterilized plate count agar solution was taken from the autoclave at 85°C and spiked on the plates. Peptone solution used for the serial dilution was prepared by dissolving 1.5 mL peptone in 1000 mL nano pure water followed by sterilization in an autoclave.

Also, 1 mM methylmercurychloride (CH_3HgCl) solution was prepared by dissolving 12.5 mg CH_3HgCl in 50 mL methanol and this was further diluted to 20 μM CH_3HgCl.

Bacterial isolates: Bacterial isolates used in this study were previously isolated from coastal marine sediment contaminated with organometallic compounds, particularly TBT compounds. The bacterial strains employed in this study was isolated from sediment slurry sample previously collected at 0-15 cm depth from Bowling Basin (Forth and Clyde Canal), Glasgow, UK (NS 450 735) in April 2009. All isolates highly resistant to TBT were used in this study to determine their sensitivity or tolerance to MeHg. Their biochemical characteristics have been studied for proper identification (Sakultanitimetha *et al.*, 2009). The TBT-highly resistant bacteria isolated are *Alcaligenes faecalis, Citrobacter braakii, Enterobacter cloacae* and *Pseudomonas fluorescens* (Inoue *et al.*, 2000). Each of these bacteria strains are inoculated in a nutrient media for bacterial growth before exposure to toxicant.

Resistance of bacteria using EC_{50}: The ability of bacteria highly resistant to TBT to grow in the presence of MeHg was tested by adding CH_3HgCl at different concentrations in a growth media. Glycerol medium was prepared by adding 1 g of K_2HPO_4, 1 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$, 0.4 g of $MgCl_2$,0.125 g of yeast extract and 1 mL of glycerol in 1 L of nano pure water. The media is subsequently sterilized in an autoclave machine prior to any further use. Two set of media were prepared for the experiment, one for the inoculation of the bacteria samples and the other for addition of the stock solution of MeHg in Methanol. The media prepared was sterilized in an autoclave prior to inoculation of bacteria and subsequently incubated at 28°C shaken at 150 rpm overnight.

Furthermore,10 mL of each sample containing MeHg was prepared by adding appropriate quantity of 20 μM CH_3HgCl stock solution in methanol to make up different concentrations 0, 0.1, 0.2, 0.3, 0.4 and 0.5 μM CH_3HgCl. Five hundred micriliter of cultured bacteria samples are enriched by inoculation into 10 mL of the media containing the stock solution with varying concentrations as outlined above. These samples were immediately incubated at 28°C shaken at 150 rpm for 7 days as considerable period of time is required for the bacteria to grow and fully acclimatized with the alien environment. After incubation for 7 days, the samples are serially diluted in a nano pure

water containing peptone. Aliquots of 100 μL of 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5},10^{-6} and 10^{-7} dilutions are spread onto a plate count agar in duplicates and are all incubated at 28°C overnight. Colonies of the bacteria within this range i.e., 20-200 were counted in order to determine the EC_{50} (McNaught and Wilkinson, 1997). Reed and Muench method was employed in performing the EC_{50} test (Reed and Muench, 1938).

Screening test for biodegradability: The screening medium used in this experiment was glycerol medium, made up of 1 g of K_2PO_4,1 g of KH_2PO_4 ,1 g of $(NH_4)_2SO_4$,0.4 g of $MgCl_2$,0.125 g of yeast extract and 1 mL of glycerol in 1 L of distilled water. Then similar glycerol media were also prepared but now with different quantity of glycerol 1000,100,10,0,0,0 μL ; thus making up to six samples of 50 mL each. The samples are adjusted to pH 6.8 by adding the required volume of 2 N NaOH prior sterilization. The study bacteria are inoculated in the samples. Thereafter, 750 μL of 20 μM CH_3HgCl stock solution in methanol was added to 50 mL of the samples to make up a final concentration of 0.3 μM MeHg. The sample containing 1 mL of glycerol was first incubated at 28°C shaken at 150 rpm overnight. One hundred microliter was streaked on plate count agar in duplicates while 4 mL was transferred into the next sample containing 100 μL of glycerol. Subsequently, it is incubated at 28°C shaken at 150 rpm overnight. Thereafter 100 μL was also streaked on the plate agar count in duplicates. This procedure was repeated for the other four samples. These procedures were adopted with slight modifications as described by Sakultanitimetha *et al*. (2009).

RESULTS AND DISCUSSION

Resistance of heterotrophic bacteria: The bacterial isolates show variation in resistance to MeHg. Colonies formations were observed 24 h after incubation in all except for *Alcaligenes faecalis* which took 48 h before colonies were counted. MeHg concentrations which inhibited colony formation by 50% (EC_{50}) were calculated by the method of Reed and Muench. The EC_{50} results for the isolates are shown in Fig. 1. Clearly, result reveals that *Enterobacter cloacae* with the highest EC_{50} value of 0.39 μM have the highest resistance to MeHg while *Pseudomonas fluorescens* with the lowest EC_{50} is the least resistant to MeHg. Notably, *Enterobacter cloacae* and *Citrobacter braakii* shows a better bacterial response by exhibiting rapid growth within 24 h while the other two takes about 48 h before noticeable colony formation were observed. This depicts the bacteria behavioural properties and activity in the medium. The former were happy with the toxicant while the latter were quite unhappy in terms of ease of growth rate. These microorganism also exhibit different morphological shapes which are unique to each bacteria isolates in terms of their biochemical characterizations.

Biodegradation potentiality: Not all microorganisms isolated from marine or freshwater sediment that show resistance or sensitivity to a xenobiotic can also degrade it. Most especially, in this scenario where the bacterial isolates used were not taken from a MeHg-contaminated sediment but TBT-contaminated sediment. However, results from the experiment shows that these bacteria isolates were able to degrade MeHg. This was confirmed by the bacteria growth (in colonies) on PCA plates observed for each samples at three successive days interval. Bacteria growth was observed in all sample media prepared, however, Table 1 shows their degree of relative turbidity (i.e., the cloudiness of the media solution).

The number of plus signs denotes the relative turbidity of the growth medium containing 0.3 μM for all bacteria strains.

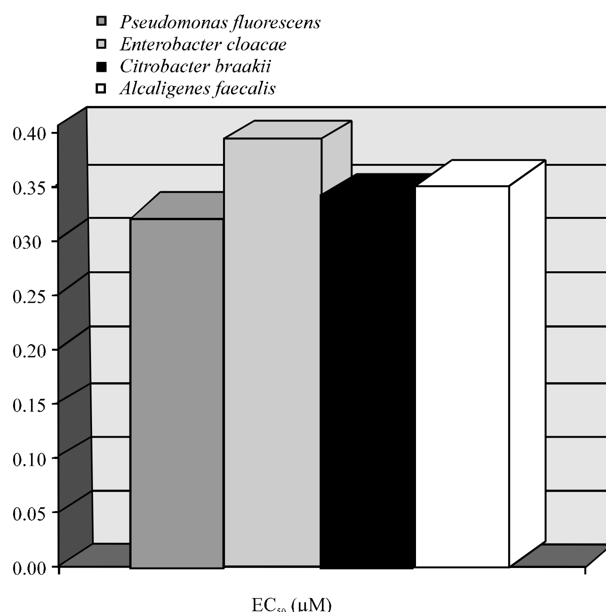

Fig. 1: Effective concentrations (EC_{50}) of isolated bacteria

Table 1: Growth of microorganisms in nutrient media as a function of time

	Incubation time (days)					
	3	6	9	12	15	18
	Amount of glycerol present in the nutrient media (µL)					
Bacteria strains	1000	100	10	0	0	0
Pseudomonas fluorescens	+++++++	+++++++	+++++++	++++	+++++++	+++++
	+++++++	+++++++	+++++++	++++		
Enterobacter cloacae	+++++++	+++++++	+++++++	++++++	+++++	+++
	+++++++	+++++++	+++++++			
Citrobacter braakii	+++++++	+++++++	+++++++	+++++	++++	++++++
	+++++++	+++++++	+++++++	+++++	+++	
Alcaligenes faecalis	+++++++	+++++++	+++++++	+++++++	++++	+++++++
	+++++++	++++++	+++++++	+++++++	++++	

DISCUSSION

Tolerance of bacteria isolates to MeHg: Microorganisms have been reported to have the capability of resisting xenobiotics and other heavy metals, particularly in natural environment where mixed pollutants exist (Agarry *et al*., 2010; Sakultanitimetha *et al*., 2009; Wuertz *et al*., 1991). From this study, TBT-resistant bacteria have been shown to be tolerant to MeHg. The result presented in Fig. 1 shows that the EC_{50} s of the selected bacteria-*Pseudomonas fluorescens*, *Enterobacter cloacae*, *Citrobacter braakii* and *Alcaligenes faecalis* to be 0.32, 0.39, 0.34 and 0.35 µM, respectively. It can be inferred that the variation of the EC_{50} values depends on the nature of the medium, biological properties of the bacteria isolates. In natural environment, other factors such as the salinity, pH, temperature, presence of other xenobiotics and heavy metals contribute to the sensitivity or resistivity of these microorganisms. Wuertz *et al*. (1991) reported that EC_{50} of TBT in freshwater is higher than in estuarine sediments.

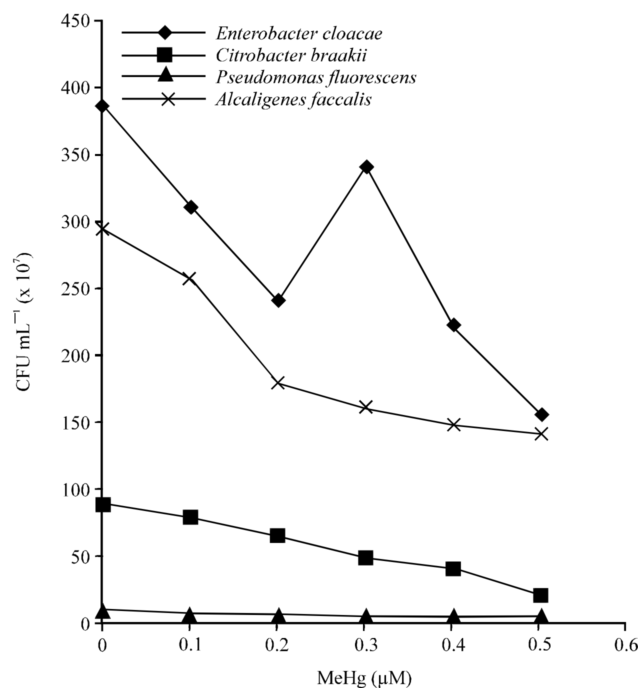

Fig. 2: Bacterial growth of the four selected isolates in nutrient media containing MeHg

Furthermore, EC_{50} values are a measure of the resistivity or tolerance of a microorganism to a particular toxicant or mixed toxicants. The lower the EC_{50}, the greater the sensitivity of the bacteria and thus the less effective it is at withstanding the toxicity of the pollutant. From Fig. 1 and 2, it was observed that that *Pseudomonas fluorescens* shows a greater sensitivity to MeHg relative to others. This observation agrees with findings reported by Shariat *et al.* (1979). Therefore, tolerance level exhibited by these bacteria strain is in the decreasing order; *Enterobacter cloacae> Alcaligenes faecalis>Citrobacter braakii>Pseudomonas fluorescens*. From Fig. 2, it could be clearly seen that both Enterobacter *cloacae and Alcaligenes faecalis* have high bacterial population which in measured in terms of CFU values. The distinct feature further complements the reason for their high EC_{50} values, suggesting that these bacterial isolates possess high tolerance to MeHg relatively to others.

The MeHg contamination level in most aquatic environment is found to be estimated at 4.59 ppb (equivalent to 0.02 µM) (Horvat *et al.*, 2004). In comparison with the EC_{50} of these two bacteria species-*Enterobacter cloacae* and *Alcaligenes faecalis*, it indicates that the level of MeHg contamination in environment would not cause major inhibition of bacteria growth (or suppress its activity) and degradability even in mixed polluted sediments.

However, it is noteworthy comparing the EC_{50} values of these bacteria with respect to two different pollutants i.e., TBT and MeHg respectively. For *Enterobacter cloacae* and *Citrobacter Braakii*, their EC_{50} values in TBT are 200 and 280 times higher than MeHg when compared with results reported by Sakultanitimetha *et al.* (2009). This indicates that their tolerance to MeHg is relatively lower to that of TBT. We can deduce that MeHg is highly toxic than TBT. One of the reasons for this discrepancy might be linked to their chemical structure thus making such compound more bioavailable (Morel *et al.*, 1998; Agarry *et al.*, 2011). In organic chemistry, the reactivity of an organic compound decreases down the homologous series as the number of alkyl

group increase (i.e., as molecular weight increases). The bulkiness of the methyl group attached to the central metal atom coupled with increasing number of ligands causes a phenomenon known as "steric hindrance effect", thus lowers the reactivity of the compound and making it to be more hydrophobic. From literature, the toxicity of MeHg is linked to its liposolubility properties which TBT lacks (Morel *et al*., 1998; Ekpenyong *et al*., 2007). TBT is not readily liposoluble and thus it is hardly taken up by microorganism.

Notably, there have been no reports found in the literature where this experiment has been attempted. Most authors often use bacteria species isolated from a contaminated sediments to study either the tolerance or degradability potential of such isolated microorganism. Nonetheless, results of this study have demonstrated for the first time that TBT-resistant bacteria that have not been exposed to MeHg can tolerate it though at a very low concentration when compared to their normal tolerance of 10 μM when isolated from MeHg-heavily contaminated sediment (Shariat *et al*., 1979; Spangler *et al*., 1973). Therefore, it follows that these organism have genetically conferred resistance that makes them to resist both compounds and possibly degrade them. In corroboration, Pain and Cooney (1998) reported that most of the TBT-resistant bacteria are also resistant to other organometals and six heavy metals (Hg, Cd, Zn, Sn, Cu and Pb), which suggest that resistance (either plasmid or chromosomally-mediated) to variety of xenobiotics may be present in the same organism. This also suggests that these bacteria strain should be tested for other noxious organometals that are of major threat to aquatic environment. All the more, the combined toxicity from different pollutants could badly influence the growth rates of these bacterial isolates.

For MeHg, two main resistance genes are found in most Hg resistant bacteria. They are classified into two namely; narrow and broad spectrum bacteria. The narrow -spectrum bacteria possesses *mer* A -mercuric reductase- which convert Hg^{2+} to Hg^0 but cannot degrade organomercury compounds since they lack the gene for organomercurial lyase (Summers and Silver, 1978; Osborn *et al*., 1997; Ravel *et al*., 2000). However, the broad-spectrum type possesses the *mer* B-organomercurial lyase-which is responsible for the cleavage of the mercury-carbon bond. They are capable of degrading all mercury chemical species (Nakamura *et al*., 1990; Brown *et al*., 1991).

Furthermore, all the isolates tested in this study were gram-negative which was previously reported by Ravel *et al*. (2000) to be far more resistant than gram-positive strain. It therefore suggests that such environmental strains are of practical interest to microbial ecologists not only to reiterate current concepts of MeHg and TBT resistance by native microflora but also to comprehend the evolution and importance of their resistance. The bacteria strain-*Enterobacter Cloacae* exhibits the greatest resistance to Hg of any gram-negative environmental isolates (De *et al*., 2008).

In TBT, the resistance genetic mechanism is not fully understood, however it has been reported that oxidative enzymes-cytochrome P 450-common in most aquatic organism were responsible for the catalysis of the first step reaction which is the hydroxylation followed by debutylation (Sakultanitimetha *et al*., 2009; Wuertz *et al*., 1991). MeHg-resistant bacteria isolates differed in their tolerance properties (Fig. 1).Tolerance properties are measured in terms of their EC_{50}s. This implies that the efficiency of mercury detoxifying systems may varies in different Hg-resistant bacteria even as observed in this study. This finding also corroborates observations by different authors (Pahan *et al*., 1995; Ray *et al*., 1993; Sadhukhan *et al*., 1997).

Effect of medium on MeHg-resistant bacteria: The plating medium used might also affect the bacterial resistances as medium-organometal interaction may affect the metal toxicities (Wuertz *et al*., 1991; Ekpenyong *et al*., 2007). However, it is difficult to relate the metal

concentration used in agar medium to environmental concentrations. Nonetheless, the reason for this is unknown as their interaction mechanisms are yet to be unfolded (Duxdury, 1985). For instance, Wuertz *et al.* (1991) found that EC_{50}s were higher on TSA than on estuarine-salts agar the EC_{50}s on estuarine agar for the polluted estuarine site and the unpolluted site differed significantly.

Biodegradation potentiality: Most bacteria that show some degree of resistance to certain xenobiotics often at times not possess the capability of degrading them. However, even though all MeHg-degrading bacteria are all MeHg-resistant bacteria, not all MeHg-resistant bacteria are MeHg-degraders. They can either degrade them by using carbon source from the nutrient media or from the toxicant itself (Billen *et al.*, 1974; Sakultanitimetha *et al.*, 2009). Biodegradation can be effected by organisms either able to utilize the pollutant for their energy and carbon requirements or only able to modify it enzymatically without using it as a nutritional source-i.e.cometabolism (Bending and Rodriguez-Cruz, 2007; Ekpenyong *et al.*, 2007).

For MeHg, potential microbes can utilise methyl (CH_3-) group in the compound as its growth substrate with incorporation of carbon. In an instant of limited carbon content, MeHg will become a sole source of carbon for bacterial survival. The results from this study shows that selected TBT-degrading bacteria isolates were not only resistant to MeHg but also possess the tendency to degrade MeHg in the nutrient media. However, Table 1 shows a gradual decrease in the turbidity and colony formation as the amount of carbon source (i.e., glycerol) is gradually lowered systematically. This implies that the carbon source have a significant impact on the microbial metabolism and hence the degradability.

Moreover, it is evident that the bacteria isolates were able to survive even in the absence of any organic source due to the fact that they derived their energy from the toxicant .With the aid of their resistance enzymes, they were able to metabolize MeHg into methane and mercury vapour (Hg^0) (Oremland *et al.*, 1991). It took longer periods, usually more than 48hrs (an indication of extended lag phase) before bacterial growth were observed. This result shows that Enterobacter and Citrobacter exhibit faster acclimation period and bacteria response. In the absence of glycerol, we observed that all the bacteria strains except *Enterobacter cloacae* grew very happily suggesting they utilise the carbon-energy source from MeHg. However, before the nutritional source was totally withdrawn, bacteria isolates breakdown MeHg as cometabolite by relying on the utilisation of the primary substrate to metabolise the secondary substrate (which is usually the toxicant).The primary substrate which is the glycerol in the media support bacteria growth and/or cell replication. Faster growth rate was observed which is confirmed by the turbidity of the media solution and the high Coliform Forming Unit (CFU) values recorded.

Consequently, co-metabolic process will be obviously depended on the growth of the microorganism (Errecalde *et al.*, 1995). Thus, the addition of nutritional source enhances biodegradation process (Kawai *et al.*, 1998; Bernat and Dlugonski, 2006). It often happens that the addition of a pollutant suitable as an energy source in a medium induces an increase in the degradation capacity of the microbial community, because of a "sociological adaptation"(Wuhrman, 1964) due to positive selection by the pollutant whose presence favours the growth of bacterial strains able to use it.

CONCLUSION

It has been successfully demonstrated that isolated TBT-resistant bacteria were able to show a significant resistance to MeHg even though it was assumed that these organisms have not been

exposed to MeHg at any time. Interestingly, this is a very first time this was attempted and gives a new scientific approach to bioremediation technologies. *Enterobacter cloacae* and *Alcaligenes faecalis* have been shown in this study to exhibit high resistance to the toxic effects of MeHg.

Furthermore, these bacteria species were also examined for their biodegradability and it has found that they shows capability of degrading MeHg even in the absence of primary nutrient-glycerol. It can be concluded that these microorganism can utilise the carbon-source in the pollutant in order to mineralize the organic compound. Again, it invalidates previous studies which from their observation suggest that cometabolism as the possible means of microbes degrading MeHg. Again, this study has successively proven that these four selected bacteria species isolated from TBT-contaminated sediment were able of utilising the organic pollutant as a nutritional substrate to carry out mineralisation activities.

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