16S rRNA Molecular Identification of Metal Tolerant Bacteria Isolated from Electronic Waste Recycling Facility and its Heavy Metal Removal Ability

Ramasamy Rajesh Kumar[1,2] and Jae Young Cho[2]

[1]Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappali 620 024, India
[2]Department of Bioenvironmental Chemistry, Chonbuk National University, Jeonju, Jeollabuk-do, 560 756, Republic of Korea.

(Received: 23 January 2013; accepted: 04 March 2013)

The lead nitrate removal by isolated lead tolerant bacteria *Pseudomonas* sp isolated from electronic waste (e-waste) recycling facility soil was studied in a laboratory simulated environment. The bacterium was identified by biochemical and 16S rRNA molecular studies. The DNA was isolated and amplified using universal bacterial primers. The phylogeny tree suggested the isolated lead tolerant bacterium as *Pseudomonas aeruginosa.* The partial 16S rRNA sequences were submitted to NCBI Genbank and the accession number JN102340 was assigned to the submitted sequences. Various parameters were optimized for lead nitrate removal such as pH, temperature, kinetics studies and metal tolerant assay. The metal removal studies suggested that the lead tolerant *Pseudomonas aeruginosa* JN102340 able to remove lead nitrate 92% at pH 6, 89% at 35ºC , 90% after 24h incubation in the optimized pH and temperature. The bacterium *Pseudomonas aeruginosa* JN102340 tolerate and survive up to 600mg L^{-1} lead nitrate.

Key words: Bioremediation; Lead removal; *Pseudomonas aeruginosa*; Bacteria; e-waste; 16S rRNA.

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Copper, chromium, cadmium and nickel are known to be the most commonly heavy metals used and the more widespread contaminants of the environment[1-3]. Traces of these heavy metals are necessary as co-factors of enzymatic reactions, but high levels of them may cause extreme toxicity to living organisms due to inhibition of metabolic reactions. The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction reactions[4-9]. The bioremediation of heavy metals using microorganisms has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application in industry. Metal accumulative bioprocess generally falls into one of two categories, bisorptive (passive) uptake by nonliving, nongrowing biomass or biomass products and bioaccumulation by living cells[10]. Microbial survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation[11]. Bahig *et al*., (2008)[12] reported that the previous studies have shown that long term and short term stresses such as high temperature, extremes of pH

* To whom all correspondence should be addressed.
E-mail: soilcosmos@jbnu.ac.kr

or chemical pollution often result in altered metabolism, species diversity and plasmid incidence of soil bacteria populations. Bacteria are among the most abundant organism that occurs everywhere on earth. Heavy metals are increasingly found in microbial habitats due to several natural and anthropogenic processes; therefore, microbes have evolved mechanisms to tolerate the presence of heavy metals by efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration[13]. Most mechanism studied till date involves the efflux of metal ions outside the cell, and genes for tolerance mechanisms have been found on both chromosomes and plasmids. Bacteria that are resistant to and grow on metals play an important role in the biogeochemical cycling of those metal ions[13]. To understand the contamination status, Ha et al., (2009)[14] measured trace elements (TEs) in soil, air dust, and human hair collected from e-waste recycling sites (a recycling facility and backyard recycling units) and the reference sites in Bangalore and Chennai in India. Concentrations of Cu, Zn, Ag, Cd, In, Sn, Sb, Hg, Pb, and Bi were higher in soil from e-waste recycling sites compared to reference sites. For Cu, Sb, Hg, and Pb in some soils from e-waste sites, the levels exceeded screening values proposed by US Environmental Protection Agency (EPA). Concentrations of Cr, Mn, Co, Cu, In, Sn, Sb, Tl, Pb and Bi in air from the e-waste recycling facility were relatively higher than the levels in Chennai city. High levels of Cu, Mo, Ag, Cd, In, Sb, Tl, and Pb were observed in hair of male workers from e-waste recycling sites. Ha *et al*., (2009)[14] results suggest that e-waste recycling and its disposal may lead to the environmental and human contamination by some TEs. This is the ûrst study on TE contamination at e-waste recycling sites in Bangalore, India.

In this study, e-waste recycling facility surface soil was used to evaluate the sorption capacity of lead nitrate by the isolated lead metal tolerant bacteria in a laboratory simulated environment.

MATERIALS AND METHODS

Samples

Soil sample was collected from an e-waste recycling facility surface soil from Peenya Industrial Estate, Bangalore, Karnataka, India (Fig. 1) and stored at -20ºC. Soil was dried to remove the moisture content.

Preparation of metal adsorbates

Different metal concentrations were prepared by dissolving Lead nitrate, in double distilled water to get metal concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg L^{-1}. A stock solution of 1000 mg L^{-1} was prepared; all other concentrations are obtained from it. Prior to experiment all the glasswares were treated with 0.1 M HCl before and after the biosorption experiments to avoid binding of metals to it. Lead nitrate was estimated using PAR Incidicator method and absorbance was measures at 520 nm.

Enumeration and screening of metal tolerant bacteria

Lead metal tolerant bacterial (MTB) strains were isolated from the soil samples using bacterial medium. Approximately 10 g of soil sample was serially diluted with peptone water and 100 ¼l of suspension from 10^{-4} dilutions from the broth was spreaded on to the nutrient agar plates (standard spread plate technique) amended with different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg L^{-1}) lead nitrate to isolate MTB strains. The plates were incubated at room temperature (30–35ºC) for 2-3 days. After incubation, the numbers of colonies were counted. Heavy metal resistant bacteria were screened by selecting the metal tolerant identical colonies from metal amended nutrient agar plate. The isolated colonies were grown in LB broth and these colonies were further characterized and employed for heavy metal tolerance studies. The pure cultures were isolated from the plate by inoculating the individual colonies into the sterile Luria Bertani Agar plates. The plates were incubated at 37ºC for 24 h.

Identification of the isolated MTB strains

Morphological, physiological, and biochemical characteristics of the isolated MTB strains were performed by following tests according to Bergey's manual[15] of systematic bacteriology.

Molecular identification of MTB by 16S rRNA

The ready to amplify DNA was isolated from the MTB by using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research Corporation, USA) by following the manufacturer's instructions. Isolated MTB DNA was run on 0.8% Agarose Gel

Electrophoresis to check the presence of DNA. MTB 16S rRNA genes were amplified by using the Universal primers (8 F' 5'-AGAGTTTGATCCT GGCTCAG-3'; 1492 R' 5'-TACGGCTACCTTG TTGTTACGACTT-3'). PCR was performed using the following reaction mixtures: 1µl of DNA, 17µl of 1x Prime Taq™ DNA polymerase (Genet Bio, Republic of Korea), 1µl of each primer (Genei, Bangalore, India), to give a ûnal volume of 20 µl. Thermal cycling was carried out under the following conditions: Denaturation at 94ºC for 3 min, Annealing at 92ºC for 1 min, 52ºC for 1 min, 72ºC for 1.30 min followed by 30 cycles of 72ºC for 5 min. The amplified MTB DNA was run on 1% Agarose Gel electrophoresis. The product was eluted and purified using HiYield™ Gel/PCR Large DNA Extraction kit (Real Biotech Corporation, Taiwan) by following the instructions manual. The purified MTB 16S rRNA was sequenced at Ocimum Biosolutions, Hyderabad, India. The MTB 16S rRNA gene sequences were initially analyzed using the BLASTn search facility. Phylogenetic tree was constructed using the software MEGA verison 5.0. The sequences were submitted to the NCBI (National Centre for Biotechnology and Information) GenBank for obtaining accession numbers.

Biosorbent quantification

The MTB biosorbents was quantified by withdrawing 2.5 ml of broth culture from the bacterial medium and the absorbance was measured using spectrophotometer at 595 nm[16]. Uninoculated growth medium was used as blank.

Optimization of pH for heavy metal removal

The MTB biosorbents was inoculated into a series of 250 ml conical flasks containing heavy metal (100 mg L^{-1}) at different pH (2, 4, 6, 8 and 10) and kept in orbital shaker (120 rpm) for 24 hrs incubation. After 24 h incubation, the biosorbent were separated by centrifugation at 3,000 rpm for 15 min and the heavy metal concentration was determined spectrophotometrically. The initial and the final concentration of heavy metal used in batch mode studies were calculated by estimating the concentration of heavy metal spectrophotometrically. From the difference in concentration the removal efficiencies of the biosorbent was calculated. Based upon the heavy metal removal and biosorbent data, the optimum pH was determined. To avoid precipitation of the heavy metal ions at high pH, all experiments were carried out only upto pH 10. The use of buûers was avoided to eliminate unknown eûects of their components in the presence of metallic ions[17]. The pH was adjusted with 1 N NaOH and 1 N HCl as required.

Optimization of temperature for heavy metal removal

The MTB biosorbents was inoculated into a series of 250 ml conical flasks containing different heavy metals (100 mg L^{-1}). The flasks were incubated at different temperatures (25, 30, 35, 40 and 45ºC) and kept in orbital shaker (120 rpm) for 24 hrs incubation. After 24 hrs incubation, the biosorbent were separated by centrifugation at 3,000 rpm for 15 min and the heavy metal concentration and bacterial biosorbent were determined by spectrophotometrically. From the heavy metal removal and biosorbent data, the optimum temperature was determined.

Measurement of the kinetics of broth cellular growth and heavy metal removal

The MTB bisorbent was inoculated into a 250 ml conical flask containing heavy metal (100 mg L^{-1}). The flask was kept in orbital shaker (120 rpm) at optimum pH and temperature for 24 hrs. During the incubation period, heavy metal concentration and biosorbent were monitored for every two hours interval (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hrs) until heavy metal removal attains a saturation level.

Heavy metal tolerance assay

Bacterial isolates were added into a 250 ml flask containing nutrient broth amended with heavy metal (ranging from 100 to 1000 mg L^{-1}). The biosorbents were shaken in a rotary shaker (120 rpm) in a temperature controlled water bath at pH 7 and 35ºC for 24 hrs. After 24 hrs incubation, the bisorbent was measured. The extent of tolerance was compared and the "normalized" bisorbent was calculated, i.e., biomass at each heavy metal concentration per biosorbent using a control. Bacterial biosorbent were quantified by spectrophotometer at 595 nm.

All the experiments were carried out in triplicates.

RESULTS AND DISCUSSION

Heavy metal quantification

A Standard graph was plotted against the concentration of standard lead and absorbance. From the standard graph, the concentration of the sample can be calculated (Fig 2).

Enumeration and screening of MTB

The population of MTB was reduced due to the increasing concentration of metals (Table 1) that exerted more stress in the medium. Brown et al., (1994)[18] concluded that the microbes continued to be metabolically active in the presence of higher concentration of heavy metals, but the number might be reduced. In general the maximum number of population was observed under control and as the concentration of heavy metals increased from 0 to 100 ppm, the population decreased sharply in all the isolates.

Identification of the isolated MTB strains

To determine the genus to which the strains belong, a series of biochemical tests were performed. Morphological, physiological, biochemical profile was subsequently examined according to Bergeys' manual, the isolated potential organisms identified which is shown in Table 2.

Identification of MTB strain by 16S rRNA

The DNA of lead tolerant bacterial strain *Pseudomonas* sp was amplified with primers 1492R and 27F. The PCR amplified products were detected by 0.8% agarose gel electrophoresis with ultraviolet (UV). The length of object fragment is about ~1, 500 bp. Sequence analysis of the 16S rRNA gene has been considered a fast and accurate method to identify the phylogenic position of bacteria. Partial 16S rRNA of *Pseudomonas* sp were sequenced and used to construct phylogenetic development trees (Fig 3). Comparative analysis of the sequences with already available database showed that the strains were closed to the members of genus and it was classified in the branch *Pseudomonas aeruginosa*. The accession number JN102340 was assigned by NCBI Genbank.

Optimization of pH for heavy metal removal by MTB strains

Hydrogen ion concentration in the adsorption is considered as one of the most important parameters that influence the adsorption behavior of metal ions in aqueous solutions[19]. It affects the solubility of the metal ions in the solution, replaces some of the positive ions found in the active sites and affects the degree of ionization of the adsorbate during the reaction[20]. Tests were conducted with initial heavy metal levels of 100 mg L^{-1}, at a temperature of 35ºC and equilibration time of 24 hrs. The effect of pH on the biosorption of heavy metal onto MTB strain was evaluated within the pH range of 2-10. *Pseudomonas aeruginosa* JN102340 reported a maximum removal of 92% at pH 6 at a concentration

Table 1. Enumeration of lead resistant bacterial species at different concentrations

Concentration of lead (mg L^{-1})	No. of Colonies	Colony forming unit (CFU/ml)
0	TNTC	-
10	TNTC	-
20	TNTC	-
30	TNTC	-
40	TNTC	-
50	32	32×10^4
60	29	29×10^4
70	16 (TFTC)	16×10^4
80	11 (TFTC)	11×10^4
90	9 (TFTC)	9×10^4
100	4 (TFTC)	4×10^4

Table 2. Morphological, physiological and biochemical characteristics of the isolated bacterial species

Morphological/Physiological /Biochemical characteristics	Isolated Lead MTB strain
Gram's staining	-
Cell shape	Rod
Indole production test	-
Methyl red test	-
Voges- Proskauer test	-
Citrate test	+
Triple sugar iron test	K/A
Gas production	-
Urease test	-
Motility test	-
Catalase test	+
Oxidase test	+
ONPG test	-
Nitrate test	+
Gelatin hydrolysis test	+
Starch hydrolysis test	-
Strain name	*Pseudomonas* sp

of 100 mg L^{-1}. The influence of pH on the percentage sorption of heavy metals by *Pseudomonas aeruginosa* JN102340 was depicted in the Fig 4. This pH dependency of biosorption efficiency could be explained by the functional groups involved in metal uptake and metal chemistry. Above pH 5, the percent removal of lead nitrate increased rapidly by the isolated *Pseudomonas aeruginosa* JN102340 strain.

The low bioaccumulation capacity at pH values below six is attributed to the competition of hydrogen ion with metal ion on the sorption site. Thus, at lower pH, due to the protonation of binding site resulting from high concentration of proton, negative charge intensity on the site is reduced which results in the reduction or inhibition for the binding of metal ion. Most of the microbial surfaces are negatively charged due to the ionization of functional group, thereby contributing to metal binding. At low pH, some of the functional groups will be positive charged and may not interact with metal ions[21]. The increase in percent removal of metal with increase in pH from two to ûve is due to the strong relations of bioaccumulation to the number of surface negative charge, which depends on the dissociation of functional group[22]. As the pH is increased above the zeta potential of the biosorbent, there is a

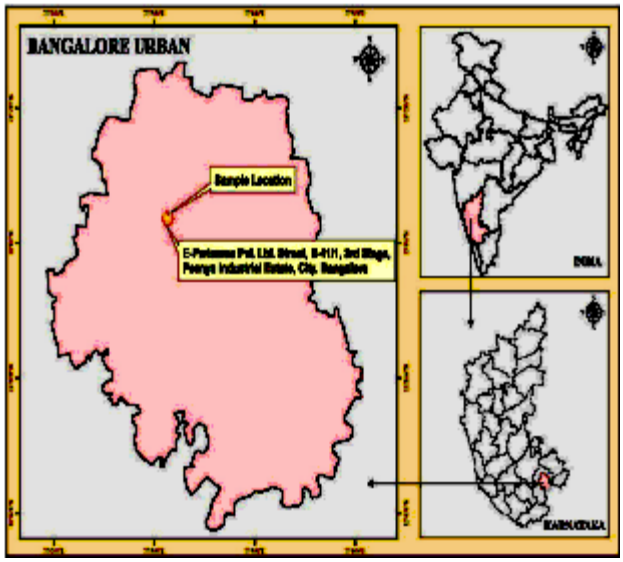

Fig. 1. Location of soil sampling: GIS map of soil sampling site at e-waste recycling facility

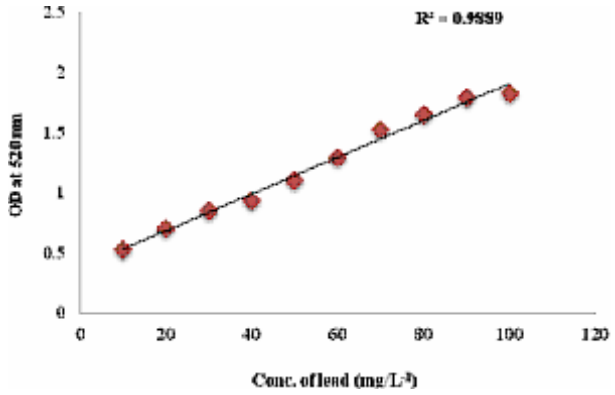

Fig. 2. Calibration of Lead (PAR Indicator Method)

reduction in the electrostatic attraction between the heavy metals and the biosorbent surface, with a consequent decrease in percentage bioaccumulation. The rate of metal uptake and the extent were enhanced as the pH increases up to certain pH range. At low pH negligible removal of metals ions noted may be due to the competition between hydrogen and metal ions. With further increase in pH, there is an increase in metal removal, which may be due to the ionization of functional groups and an increase in the negative charge density on the cell surface. At higher alkaline pH values (8 and above), a reduction in the solubility of metals contributes to lower uptake rates.

Optimization of temperature for metal removal by MTB species

The range of optimal temperature values (30–35ºC) were comparable to the range of room temperature that was used when isolating the microorganisms, suggesting that the selection of these isolates might have been inûuenced not only with the heavy metals but also with the temperature used in the isolation procedure. The temperature of the adsorption medium could be important for energy dependent mechanisms in metal removal by microorganisms. Temperature is known to affect the stability of the cell wall, its conûguration and can also cause ionization of chemical moieties. These factors may simultaneously affect the binding sites on isolated fungal and bacterial species causing reduction in heavy metal removal. Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature[23]. Bioaccumulation of metal ions MTB species appears to be temperature dependent.

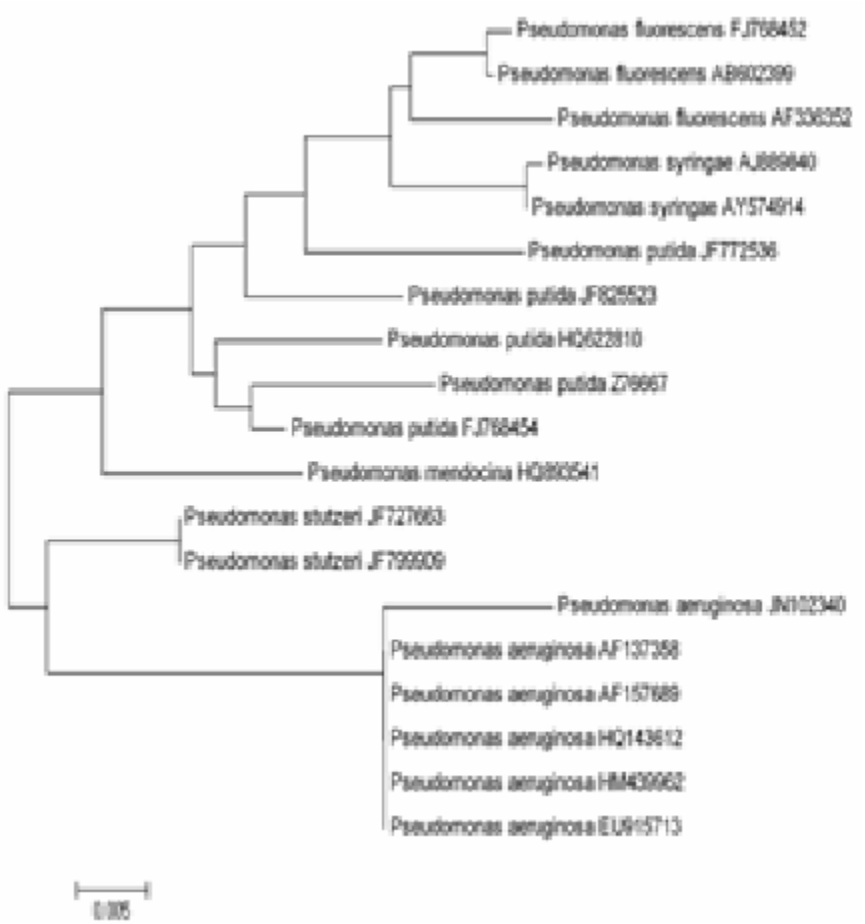

Fig. 3. Neighbor-Joining tree deduced from partial sequences of 16S rRNA gene of lead MTB *Pseudomonas* sp EWRR4 isolated from the e-waste recycling facility surface soil

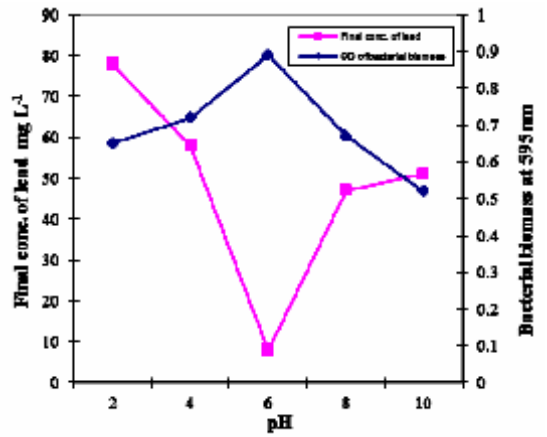

Fig. 4. Cellular growth and lead removal by *Pseudomonas aeruginosa* in response to various pH

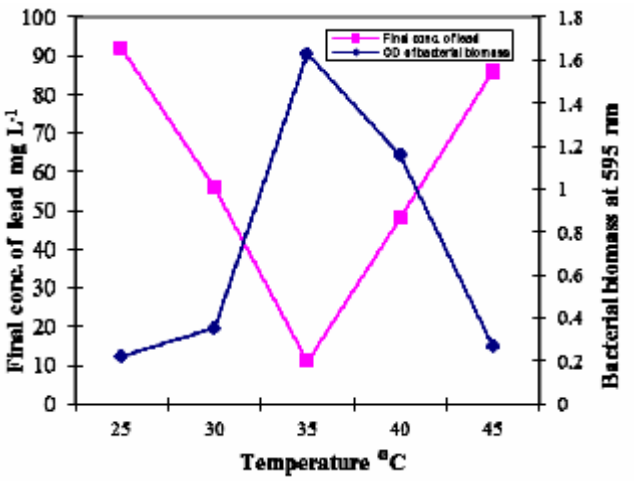

Fig. 5. Cellular growth and lead removal by *Pseudomonas aeruginosa* in response to various temperatures

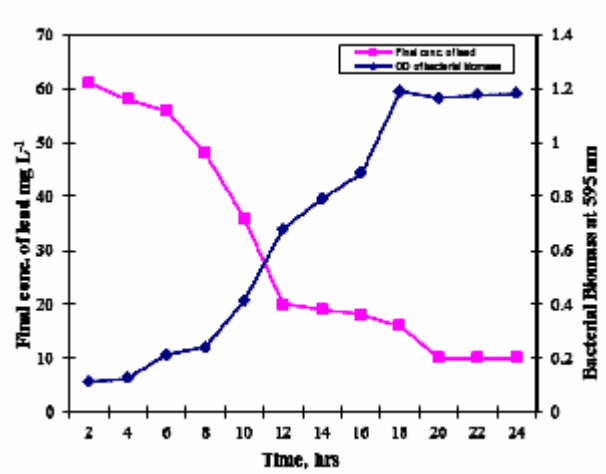

Fig. 6. Kinetics of cellular growth and lead removal by *Pseudomonas aeruginosa*

Maximum removal of lead nitrate by *Pseudomonas aeruginosa* JN102340 was observed at 35ºC. The temperature of the adsorption medium could be important for energy dependent mechanisms in metal biosorption. Energy-independent mechanisms are less likely to be affected by temperature, since the processes responsible for biosorption seems to be largely physicochemical (electrostatic forces) in nature[24]. A lot of experimental studies had been done on effects of temperature on heavy metal removal by microbes, and the researches were reported on effects of different microbial species and conditions[25, 26], temperature affects biosorption of heavy metals and bacterial acitivity as well. To study the effect of temperature on heavy metal removal, we conducted tests with different equilibration temperatures. Initial heavy metal levels are 100 mg L^{-1} for all four heavy metals. Experiments was carried out at pH 6.0 for 24 h and the result was shown as Fig. As indicated by Fig 5, when the equilibration temperature ascended from 25ºC to 35ºC, bacterial biomass and removal of lead nitrate also increased with temperature and afterwards declined when temperature became even higher. Therefore, the best temperature for maximum removal of lead nitrate by *Pseudomonas aeruginosa* JN102340 is 35ºC and the removal was 89%.

Kinetics of cadmium removal and cellular growth of the MTB species

Experiment was conducted to investigate heavy metal removal courses and the result was shown as Fig 6. It was found from the Fig that within 24 hrs *Pseudomonas aeruginosa* JN102340 could remove 90% of lead nitrate. After 12 hrs, bacterial biomass of *Pseudomonas aeruginosa* JN102340 and removal of lead nitrate also increased very slightly with time and removal equilibrium. The time-course data for heavy metal removal and cellular growth were observed for each isolate under optimal pH and temperature conditions. When these isolates are applied in removing heavy metal from industrial wastewater, information regarding the effect of growth phase will be important in designing solid (sludge) retention time (SRT) for continuous ûow completely stirred (CFCS) bioreactor, which is a general reactor type for wastewater treatment plants[16]. In the MTB isolates, speciûc metal bioaccumulation (accumulative biosorption (removal) of each heavy metal per accumulative biomass) increased when cells were in stationary phases. Therefore, expanded SRTs (stationary phase) may be recommended using the *Pseudomonas aeruginosa* JN102340 isolates in removing heavy metals from industrial wastewater however, a non-expanded SRT has to be designed for CFCS bioreactor so that a mid-log phase of cellular growth could be kept in the treatment system. The growth rate during the lag phase was very low because the isolated *Pseudomonas aeruginosa* JN102340 was adapting with the environment. After this stage, the isolates grew in logarithmic form using the nutrients

Influence of initial metal concentration

The initial concentration of the lead nitrate in the solution remarkably influenced the equilibrium uptake. It was noticed that initial concentration increased the sorption of heavy metal as is generally expected due to equilibrium process. This increase in uptake capacity of the *Pseudomonas aeruginosa* JN102340 with the increase in initial lead metal concentration is due to higher availability of metal ions for the sorption. Moreover, higher initial concentration provides increased driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phase resulting in higher probability of collision between metal ions and sorbents. This also results in higher metal uptake [27].

Tolerance implies a large change in sensitivity between sets of organisms to a particular toxicant. Tolerance can be adaptive, constitutive, or induced. Adaptive tolerance is where the organism colonizing a contaminated site is less insensitive than the same species colonizing uncontaminated sites and where this change in sensitivity is caused by the selection of genes that confer enhanced insensitivity. Induced tolerance, for which there is less evidence, is where particular enzymes that cause decreased sensitivity are induced on exposure to metal ions[28].

Pseudomonas aeruginosa JN102340 exhibited growth even at higher levels of metal ions and the biomass production decreased with increase in the metal concentration. A significant reduction of mean growth was achieved in all the *Pseudomonas aeruginosa* JN102340 with the

increasing concentration of heavy metals in the medium. Konopka et al., (1999)[29] confirmed that the microbial biomass generation was decreased as the concentration of heavy metal increased. This is in agreement with the findings of Hussein et al., (2004)[30] who reported that the total amount of biomass production decreased while increasing the concentration of heavy metals. Bridge et al., (1999)[31] also confirmed that the microorganisms can release a diverse range of specific and nonspecific metal binding compounds in response to high levels of toxic metals which can ameliorate the effect of toxic metals and mediate the uptake process.

CONCLUSION

The present study evaluated and suggested that the lead metal tolerant bacterium *Pseudomonas aeruginosa* JN102340 can effectively remove lead nitrate in an optimized conditions. Lead metal ion uptake by *Pseudomonas aeruginosa* JN102340 concede to look into process using this biomass for removing metal ions from industrial waste waters.

ACKNOWLEDGEMENTS

This research was supported by "Cooperative Research Program for Agriculture Science & Technology Development" Rural Development Administration, Republic of Korea (Project No.: PJ0083962013)

REFERENCES

1. Patterson, JW. Wastewater treatment technology. Ann Arbor science Publishers. 1977; Ann Arbor, MI, USA.
2. Aksu, Z. Biosorption of heavy metals by microalgae in batch and continuous systems. In: Algae for waste water treatment. eds Y.-S. Wong and N. F. Y. Tam, 1998; **99**:37-53. Springer, Germany.
3. Doenmez, G., and Aksu, Z. The effect of copper (II) ions on the growth and bioaccumulation properties of some yeasts. *Process Biochem*., 1999; 35: 135-142.
4. Rai LC., Gaur JP., and Kumar HD. Phycology and heavy metal pollution. *Biol Rev*., 1981; **56**: 99-151.
5. Macaskie L and Dean ACR. Microbial metabolism, desolubilisation and deposition of heavy metals: Metal uptake by immobilised cells and Appication to the detoxification of liquid wastes. *Adv Biotechnol Proc.,*1989; **12**: 159-172.
6. Huang, C., Huang, C., and Morehart, A.L. The removal of copper from dilute aqueous solutions by *Saccharomyces cerevisiae*. *Water Res*., 1990; **24**: 433-439.
7. Brady, D. and Duncan JR. Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Sccharomyces cerevisiae*. *Biotechnol Bioeng.,* 1994; **44**: 297-302.
8. Brady, D., Stoll, A.D., Starke, L., and Duncan, J.R. Bioaccumulation of metal cations by *Saccaromyces cerevisiae*. *App Microbiol Biotechnol*., 1994; **41**: 149-154.
9. Veglio, F., Beolchini, F., and Gasbarro, A. Biosorption of toxic heavy metals: an equilibrium study using free cells of *Arthrobacter* sp. *Process Biochem.,* 1997; **32**: 99-105.
10. Clausen, CA. Isolating metal-tolerant bacteria capable of removing copper, chromium, and arsenic from treated wood. *Waste Manage Res.,* 2000; **18**: 264-268.
11. Wuertz, S. and Mergeay, M. The impact of heavy metals on soil microbial communities and their activities. In: van Elsas, J.D., Trevors, J.T., Wellington, E.M.H. (Eds.), Modern Soil Microbiology. Marcel Dekker, New York, pp.1997; 607-639.
12. Bahig, A.E., Aly, E.A., Khaled, A.A., and Amel, K.A. Isolation, characterization and Appication of bacterial population from agricultural soil at Sohag Province, Egypt. *Malay J Micro.,* 2008; **4**: 42-50.
13. Gadd, G.M. In: Microbial Mineral Recovery (Ehrlich, HL and Brierley, CL., Eds.), McGraw-Hill, New York, pp. 1990; 249-275.
14. Ha, N.N., Agusa, T., Ramu, K., Tu, N.P.C., Murata, S., Bulbule, K.A., Parthasarathy, P., Takahashi, S., Subramanian, A., and Tanabe, S. Contamination by trace elements at e-waste recycling sites in Bangalore, India. *Chemosphere.,* 2009; **76**: 9-15.
15. Boone, D.R., Castenholz, C.W., George M. Garrity, G.M., eds. Bergey's manual of systematic bacteriology. Vol. 1, 2d ed. New York, Springer, 2001.
16. Rajeshkumar, R., Sahu, S., and Agharwal, J.R. Biosorption of cadmium (II) ions by the cadmium tolerant bacteria isolated from the chemical exposed soil of fireworks industry. *J Pure and Appl Microbio.,* 2012; **6** (2): 781-787
17. Taniguchi, J., Hemmi, H., Tanahashi, K., Amano, N., Nakayama, T., and Nishino, T. Zinc

biosorption by a zinc-resistant bacterium, *Brevibacterium* sp. strain HZM-1. *App Microbiol Biotechnol.,* 2000; **54**: 581-588.

18. Brown, R.L., Bowman RS and Kieft TL (1994). Microbial effects on Nickel and cadmium sorption and transport in volcanic tuff. *J Environ Qual.,*1994; **23**:723-729.
19. Goyal, N., Jain, S.C. and Banerjee, U.C. Comparative Studies on Microbial Adsorption of Heavy Metals. *Adv Environ Res.,* 2003; 7: 235-250.
20. Nomanbhay, S.M. and Palanisamy, K. Removal of heavy metals from industrial wastewater using chitosan coated oil palm shell charcoal. *Electronic J Biotechnol.,* 2004; **8**: 43-53.
21. Yan, G. and Viraraghavan, T. Heavy metal removal from aqueous solution by fungus *Mucor rouxii*, *Water Res.,* 2003; **37**: 4486-4496.
22. Yakup, A.M., Gulay, B., Meltem, Y., Sema, B., and Omer, G. Biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} by Ca alginate and immobilized wood-rotting fungus *Funalia trogii. J Hazard Mater B.*, 2004; **109**: 191-199.
23. Gulay, B., Sema, B., and Yakup. A.M. Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor. J Hazard Mater.,* 2003; **10**: 285-300.
24. Scott, J.A. and Palmar, S.J. Cadmium biosorption by bacterial exopolysaccride. *Biotech Lett*., 1988; **10**(11): 21-24.
25. Bengtsson, L., Johanson, B., Hackett, T.J, et al. Studies on the biosorption of uranium by *Talarmyces emersonii* CBS814.70. *App Microbiol Biotechnol.,* 1995; **42**: 807- 811.
26. Wu, J., Li, Q.B., Deng, X., et al. Studies on biosorption of Pb^{2+} by *Phanerochate chrysosoporium. Acta Microbilogica Sinica.,* 1999; **39** (1): 87 - 90.
27. Tewari, N., Vasudevana, P. and Guhab, B.K. Study on biosorption of Cr (VI) by *Mucor hiemalis. Biochem. Eng. J.,* 2005; **23**:185-192.
28. **Meharg, A.A.** The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations. *Mycol Res.,* 2003; **11**:1253-1265
29. Konopka, A., Zakharova, J., and Bischoff, M. Microbial biomass and activity in lead contaminated soil. *App Environ Microbiol.,* 1999; **65**: 2256-2259.
30. Hussein, H., Ibrahim, S.F., Kandeel, K., and Moawad, H. Biosorption of heavy metals from waste water using *Pseudomonas* sp. *Electronic J Biotechnol.,* 2004; 7(1): 38-46.
31. Bridge, T.A.M., White, C., and Gadd, G.M. Extra cellular metal binding activity of the sulphate-reducing bacterium *Desulfococcus multivorans. Microbiol.,* 1999; **145**:2987-2995.