International Journal of Theoretical & Applied Sciences, 10(2): 28-32(2018)

ISSN No. (Print): 0975-1718
ISSN No. (Online): 2249-3247

Growth Kinetics of Mercury Resistant *Pseudomonas aeruginosa*, *Lysinibacillus fusiformis and Serratia marcescens*

Sneha Bajpai and J.L. Bhat
Department of Life Science,
ITM University Gwalior (Madhya Pradesh), India

(Corresponding author: Sneha Bajpai)
(Received 20 April, 2018 accepted 27 July, 2018)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Mercury is most hazardous and toxic pollutant. The three bacteria isolated from soil samples were taken from thermal power project Jhansi (U.P.) and Bharat Heavy Electrical Limited Jhansi (U.P.) were identified by morphology and molecular (16SrRNA) studies as *Lysinibacillus fusiformis, Pseudomonas aeruginosa* and *Serratia marcescens*. Growth of isolates was assessed in mercury from 10µg/ml Hg^{++} to 100 µg/ml Hg^{++} at temperature of 30ºC and pH 6-7. The growth of isolates was decreased and extent of reduction was dosage dependent. Growth of isolates decreased with increase in concentrations of mercury. Lag phase extended in all treatments of mercury. Log phase was reduced in *S. marcescens*. Growth of *P. aeruginosa* treated with 10, 20, 25, 50, 75 and 100 µg/ml Hg^{++} was reduced by 55.94%, 71.32%, 73.07%, 76.92%, 85.66% and 89.86 % respectively. Growth of *L. fusiformis* was reduced by 30.25%, 38.74%, 40.95%, 56.08%, 67.15% and 69.28% by treatment of 10, 20, 25, 50, 75 and 100 µg/ml Hg^{++} respectively. Growth of *S. marcescens* was reduced by 61.57%, 65.41%, 73.80%, 77.24%, 79.36% and 83.33% by treatment of 10, 20, 25 , 50, 75 and 100 µg+/ml Hg^{++} respectively. Maximum growth occurred in *S. marcescens* and *L. fusiformis* on 30 hours of incubation and in *P. aeruginosa* on 42 hours of incubation. MIC of mercury for *P. aeruginosa* was 135µg/ml, for *L. fusiformis* was 190 µg/ml and for *S. marcescens* was 210 µg/ml.

Key Words: Mercury, Minimum inhibitory concentration, Growth kinetics, Heavy metal

I. INTRODUCTION

Mercury is toxic and most hazardous pollutant. It also occurs naturally and mercury has applications in industrial chemicals, meterological equipments, mercury vapour lamps, medicine, battery industry etc. Mercury is released into atmosphere by volcanoes, degassing from earth and also from evaporation of oceans [1]. Anthropogenic emission sources are combustion of coal and oil, domestic and industrial waste discharge. In sediments of water bodies mercury is transformed to CH_3Hg^+ which is highly toxic and bioaccumulates in tissues of fishes and humans. Biomagnification of CH_3Hg^+ seriously affects the health, the highest level of mercury is found in consumers at top of food chain [2]. Mercury poses great threat to human beings once mercury enters the human body, the damage caused is irreversible. It has high persistence [3]. It reduces water quality, reduces soil fertility, adversely effects biota including microorganisms. However some bacteria tolerate and grow in mercury rich environment [4]. Robinson and Tuovinen [5] were first to isolate the mercury resistant bacteria in Japan, since then several authors have also reported such bacteria [6-13]. The various conventional technologies to decontaminate have been found in effective, less practical and expensive. Bioremediation is an effective technique. Bioremediation by bacteria is emerging alternative promising technology to chemical treatments [14]. *Pseudomonas aeruginosa* has been used for different purposes by various workers [15-16]. The use of microorganisms to clean metal contaminated effluents is effective since it is efficient and eco-friendly. Heavy metal binds with thiol (SH) group in proteins, which causes inactivation of enzymes and membrane damage [17]. Mercury is genotoxic, and interacts with bases of DNA and damage to nucleic acids which is irreversible [18].

The aim of this research is to study the effect of mercury on growth of isolated bacteria and to evaluate the mercury resistance of these bacteria. The bacterial susceptibility was evaluated by determination of MIC.

II. MATERIALS AND METHODS

A. Sample Collection

Soil samples were taken from Parichha power project and Bharat heavy electrical limited Jhansi, U.P. (India) located at 25.44ºN and 76.56º E. Soil was collected from 8 to 10 cm depth, air dried and was stored at 4°C.

B. Isolation of bacteria

Bacteria from collected samples were isolated by serial dilution method, 0.1 ml of each diluent was spread on NA plate and incubated at 37ºC for 2 days.

The nutrient agar was supplemented with 10µg/ml of mercuric chloride and after incubation the pure colonies were picked and pure cultures prepared.

C. Screening

The isolates were cultured in LB broth medium supplemented with 25µg/ml of mercuric chloride and OD was recorded at 600 nm and based on maximum growth three strains were selected.

D. Growth assessment of selected isolates

Growth of selected isolates *P. aeruginosa*, *L. fusiformis* and *S. marcescens* was conducted in LB broth medium. Growth medium was supplemented with different concentrations of $HgCl_2$ such as 10ug/ml, 20ug/ml, 25ug/ml, 50ug/ml, 75ug/ml and 100ug/ml and was inoculated with 4ml of culture and incubated in shaking incubator at 37ºC for 2 days. Growth was observed at intervals of 6 hrs upto 48 hrs. OD was taken at 600 nm using spectrophotometer (Perkin-Elmer).

E. Minimum inhibitory Concentration (MIC)

Selected isolates were cultured in LB broth medium containing 50 µg/ml of mercuric chloride at 30ºC for 24 hours. Growth was observed by taking absorbance at 600 nm. Growth was observed at different concenteration of Hg^{++} (75-200 /ml Hg^{++}). The minimum concentration of Hg^{++} at which no growth of isolate occurred was taken as its MIC.

III. RESULTS AND DISCUSSION

A. Isolation of mercury resistant bacteria

Serial dilution method was adopted to isolate the mercury resistant bacteria. Initially 15 isolates tolerated 10 µg/ml of mercury. Further these 15 isolates were cultured in LB broth medium containing 25 µg/ml of mercury, three best isolates were identified on basis of maximum growth. The morphological, biochemical and molecular characterization (16S rRNA sequences) of these three isolates was done. The three bacterial isolates were identified as *P. aeruginosa* (LC036546), *L. fusiformis* (LC036547) and *S. marcescens* (LC036548). Their sequences were deposited in NCBI Gene Bank.

B. Effect of mercury on growth

The growth pattern of these three bacteria in presence of mercury metal is presented in Fig. 1. *P. aeruginosa* was cultured in L.B. broth medium at 30ºC and pH 6 supplemented with 10-100 µg/ml Hg^{++}. Growth of *P. aeruginosa* was reduced by mercury treatment of 10-100 µg /ml Hg^{++}. Lag phase was extended by mercury treatments and log phase was upto 42 hours.

Growth of *P. aeruginosa* treated with 10, 20, 25, 50, 75 and 100 µg/ml Hg^{++} reduced by 55.94%, 71.32%, 73.07%, 76.92%, 85.66% and 89.86 % respectively. *L. fusiformis* was cultured in L.B broth medium at 30ºC and pH 6 supplemented with 10-100 µg/ml Hg^{++} Growth of *L. fusiformis* was reduced by mercury treatment. Lag phase extended in all treatments of mercury and log phase was upto 30 hours. Growth of *L. fusiformis* reduced by 30.25%, 38.74%, 40.95%, 56.08%, 67.15% and 69.28% by treatment of 10, 20, 25, 50, 75 and 100 µg /ml Hg^{++} respectively. *S. marcescens* was cultured in L.B. broth medium at 30ºC and pH 7 supplemented with 10-100 µg/ml Hg^{++}. Growth of *S. marcescens* was reduced by mercury treatments. Lag phase was extended in all treatments of mercury. Log phase in control was upto 36 hours, while log phase was reduced to 30 hours in all treatments of mercury. The growth reduced by 61.57%, 65.41%, 73.80%, 77.24%, 79.36% and 83.33% by mercury treatment of 10, 20, 25, 50, 75 and 100 µg/ml Hg^{++} respectively.

Maximum growth occurred in *S. marcescens* on incubation of 30 hrs, in *L. fusiformis* on incubation of 30 hrs and in *P. aeruginosa* on incubation of 42 hrs. Growth of all three species of bacteria was reduced due to mercury treatment and reduction was dosage dependent. The difference in growth pattern could be due to different resistance and bioavailability of metal [19]. Increasing of mercury concentration in medium decreased cell differentiation [20]. Several scientists reported extended log phase in mercury treated *Pseudomonas species* [21].

MIC of mercury against different isolates is presented in Table 1. Mercury resistance in bacteria was first reported in *Streptomyces aureus* [22]. Mercury resistance occurs in number of bacteria, both in Gram+ve and Gram-ve bacteria. In this study MIC of mercury for *P. aeruginosa* was 135µg/ml. MIC of mercury for*P. Aeruginosa* isolate was variable [23] and was in the range of 2.7 - 86.4µg/ml. MIC of mercury for *P. aeruginosa* 0.08 - 0.1 mM [24] 10 mM [25].

In this present study MIC of mercury for *L. fusiformis* was 190 µg/ml in LB broth medium. MIC of 12mg/l mercury for L. fusiformis in LB media and 3mg/ l in PB media was reported [25]. In this present study MIC of mercury for *S.marcescens* was 210 µg/ml in L B broth. MIC of 12mg/l mercury for *S. marcescens* in LB media and 6 mg/ l in PB media was reported [26]. Bacteria have resistance to toxic metals through various mechanisms [27].

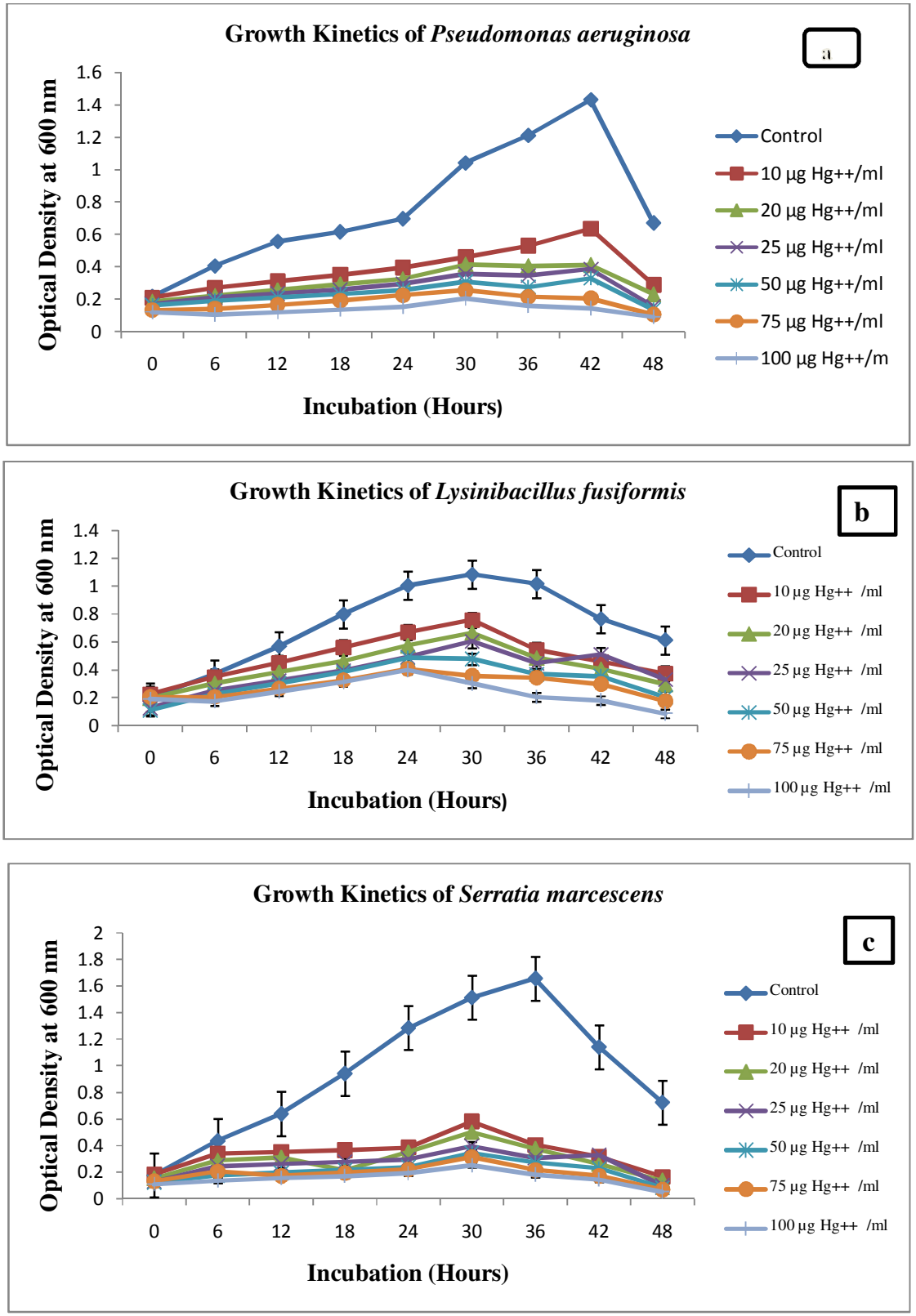

Fig. 1. Growth curve in L.B broth medium containing mercury. (a) *P. aeruginosa,* (b) *L. fusiformis* and (c) *S. marcescens*. Control cultures did not contain any Hg^{++} ion.

Table 1: Minimum inhibitory concentration of mercury against different isolates.

Bacterial Isolate	MIC Value
Pseudomonas aeruginosa	135 µg/ml
Lysinibacillus fusiformis	190 µg/ml
Serratia marcescens	210 µg/ml

These mechanisms include efflux enzyme mediated transformation, extra and intracellular transformation and formation of complex compounds with cell components [28]. Gene encoding mercury resistant (mer A) is found in many bacteria [29]. Thus it is concluded that these three bacteria will beuseful in bioremediation.

ACKNOWLEDGEMENT

We are thankful to Hon'ble Vice Chancellor, Prof. Kamal Kant Dwivedi and management, ITM University, Gwalior (Madhya Pradesh) India for all the necessary facilities for the present study.

REFERENCES

[1]. Boening, D.W., (2000). Ecological effects,transport, and fate of mercury : a general review. *Chemosphere*. **40**: 1335-1351.

[2]. Glendinning, K. J., Macaskie, L E., Brown, N L., (2005). Mercury tolerance of thermophilic *Bacillus sp.* and *Ureibacillus sp*. *Biotechnol. Lett*. **27**: 1657–1662.

[3]. Wang, Q., Kim, D., Dionysiou, D.D., Sorial, G. A., Timberlake, D., (2004). Sources and remediation for mercury contamination in aquatic systems—a literature review. *Environ. Pollutant.,* **131**: 323-336.

[4]. Iwahori, KF., Takeuchi, K., Kamimura, ST., (2000). Ferrous Iron –dependent volatalization of mercury by the plasma membrane of *Thiobacillus ferroxidans*. *Appl. Environ. Microbiol.* **66**: 3823-3827.

[5]. Robinson, J.B., Tuovinen, O.H., (1984). Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds, physiological biochemical and genetic analysis. *J. Microbiol. Rev.***48**: 95-124.

[6]. Brown, D.L., Shith, Y.C., Leang, C., Glendinning, K.J., Hobman, J.L., Wilson, J.R., (2002). Mercury transport and resistance. *Biochem. Soc. Trans.* **30**, 715-718.

[7]. Mindlin, S., Minakhin, L., Petrova, M., Kholodii, G., Minakhina, S., Gorlenko, Z., & Nikiforov, V. (2005). Presentday mercury resistance transposons are common in bacteria preserved in permafrost grounds since the Upper Pleistocene. *Res Microbiol.,* **156**: 994–1004.

[8]. De, J., Ramaiah, N., (2007). Characterization of marine bacteria highly resistant to mercury exhibiting multiple resistance to toxic chemicals. *Ecol. India.* **7**: 511-520.

[9]. Jan, A.T., Azam, M., Ali, A., Haq, QM., (2014). Molecular characterization of mercury resistant bacteria inhabiting polluted water bodies of different geographical locations in India. *Curr. Microbiol.,* **65**: 14-21.

[10]. Irawati, W., Soraya, Y., Baskoro, AH., (2012). A study on mercury-resistant bacteria isolated from a gold mine in Pongkor Village, Bogor, Indonesia. *Hayati J. Biosci*. **19**: 197-200.

[11]. Figueiredo, N . L., Canário, J., Duarte, A., Serralheiro, M.L.,Carvalho, C., (2014). Isolation and characterization of mercury-resistant bacteria from sediments of Tagus Estuary (Portugal): Implications for environmental and human health risk assessment. *J. Toxicol. Environ. Part A*, **77**: 155-168.

[12]. Mahbub, K. R., Krishnan, K., Megharaj, M., Naidu, R., (2016). Bioremediation potential of a highly mercury resistant bacterial strain *Sphingobium* SA2 isolated from contaminated soil.*Chemosphere*, **144**: 330-337.

[13]. Saranya, K., Sundaramanickam, A., Shekhar, S., Swaminathan, S., Balasubramanian, T., (2017). Bioremediation of mercury by *Vibrio fluvialis* screened from industrial effluents. *BioMed. research.* doi.org/10.1155/2017/6509648.

[14]. Brierley, C.L., (1990). Bioremediation of metal contaminated surface and ground water. *Geo Microbiology J.* **8**: 201–233.

[15]. Reeba Panesar, P.S. Panesar, D. Hasija, M.B. Bera and Harish Kumar (2009). Fermentative potential of *Pseudomonas aeruginosa* strain for biosurfactant production. *Biological Forum — An International Journal*, **1**(2): 102-105.

[16]. Janardan Yadav, Jay Prakash Verma and Kavindra Nath Tiwari (2010). Effect of plant growth promoting *Rhizobacteria* on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under in Vitro conditions. *Biological Forum — An International Journal*, **2**(2): 15-18.

[17]. Flora, S. J., Mittal, M., Mehta, A., (2008). Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J. Med. Res*. **128**: 501–523.

[18]. Sletten, E., Nerdal, W., (1997). Interaction of mercury with nucleic acids and their components. *Met. Ions. Biol. Syst.* **34**: 479.

[19]. Govarthanan, M., Park, SH., Park, Y. J., Myung, H., Krishnamurthy, R.R., Lee, SH., Lovanh, N., Kamala-Kannan, S., Oh, B.T., (2015). Lead biotransformation potential of allochthonous *Bacillus* sp. SKK11 with sesame oil cake extract in mine soil. *RSC Adv.,* **5**: 54564–54570.

[20]. Kowalczyk, A.,Willinska, M., Chyc,M., Bojko, M., Latowski, D., (2016). Isolation,screening and identification of mercury resistant bacteria from contaminated soil. SEEDE3Sweb conf.10,00042.

[21]. Ali, A., Rehman, A. (2014). Uptake of mercury by a bacterium, *Pseudomonas* sp. AN29, isolated from industrial effluents and its potential use in waste water treatment. *IJSTS*. **38**: 231-237.

[22]. Moore, B., (1960). A new screen test and selective medium for the rapid detection of epidemic strains of *Streptomyces aureus*. *Lancet*. **11**: 453–458.

[23]. Ali-Enzi, R. M., Al - Charrakh, A.H. (2013). Heavy metal resistance of *Pseudomonas aeruginosa* isolated from chemical and environmental sources in Hilla city. *Med. J . Babylon,* **10**: 110-119.

[24]. Hassen, A., Saidi, N., Cherif, M., Boudabous A., (1998). Resistance of Environmental bacteria to heavy metals. *Bioresour. Technol*. **64**: 7-15.

[25]. Haroun, A.A., Kamaluddeen, K.K., Alhaji, I., Magaji, Y., Oaikhena, E.E., (2017). Evaluation ofheavy metal tolerance level (MIC) and bioremediation potentials of *Pseudomonas aeruginosa* isolated from makera-kakuri industrial drain in Kaduna, Nigeria. *European Journal of Experimental Biology*. **5**: 2248-9215.

[26]. François, F., (2012). Isolation and characterization of environmental bacteria capable of extra cellular biosorption of mercury. *Appl. Environ. Microbiol.***78**: 1097-1106.

[27]. Silver, S., Phung, L.T., (2005). A bacterial view of the periodictable: genesand proteins for toxic inorganic ions. *J. Ind. Microbiol. Biotechnol*. **32**: 587 –605.

[28]. Nies D.H. (1999). Microbial heavy-metal resistance. *Appl. Microbiol. Biotechnol.* **51**: 730–750.

[29]. Barkay, T., Kritee, K., Boyd, E., Geesey., G.G., (2010). Athermophilic bacterial origin and subsequent constraints by redox, light and salinity on the evolution of the microbial mercuricreductase. *Environ. Microbiol.,* **12**: 2904-2917.