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JOURNAL OF ENVIRONMENTAL MICROBIOLOGY AND TOXICOLOGY

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Effect of Heavy Metals on Cyanide Biodegradation by Resting Cells of *Serratia marcescens* strain AQ07

Karamba Kabiru Ibrahim[1,2], Mohd Arif Syed[1], Mohd Yunus Shukor[1] and Siti Aqlima Ahmad[1*]

[1] Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia,
[2] Department of Microbiology, Faculty of Science, Bauchi State University, Gadau Main Campus, Bauchi State, Nigeria.

***Corresponding author:** aqlima@upm.edu.my
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia, 43400 UPM Serdang,
Selangor, Malaysia,
Tel. No: +603-89478292

HISTORY
Received: 28st October 2014
Received in revised form: 21st of December 2014
Accepted: 29th of December 2014

KEYWORDS
heavy metals
biodegradation,
Serratia marcescens
resting cells
potassium cyanide

ABSTRACT

Effect of heavy metals was tested on *Serratia marcescens* strain AQ07 (accession number: KP213291) to affirm its effect on growth and biodegradation of cyanide. 1 ppm of ten different heavy metals was experimented in-vitro in buffer medium containing 200 ppm potassium cyanide. They are: silver, arsenic, cadmium, cobalt, chromium, cupper, mercury, nickel, lead and zinc. The results obtained illustrates that mercury have significant effect on the growth (optical density 0.13) and biodegradation of cyanide removing only 24.7% compared to the control which has no incorporation of heavy metal (optical density 0.74 and 92% removal respectively) while others remove above 80%. Further studies on mercury reveal that it has significant effect on the bacteria even as low as 0.1 ppm. This illustrates that mercury can significantly hinder biodegradation of cyanide by *Serratia marcescens* strain AQ07.

INTRODUCTION

Serratia marcescens strain AQ07 was isolated from soil environment in the premise of Universiti Putra Malaysia (UPM). It was screened for cyanide degradation and found to be very effective. It produces red pigment called prodigiosin that has high sensitivity to toxicants [1]. Cyanide reacts with gold, mercury; cobalt and iron to from stable complexes which are highly stable even in mildly acidic conditions. Other complexes formed by cyanide with other metals from the ore are copper, iron and zinc. Gold extraction is usually carried out with cyanide which is a process referred to as cyanidation. Cyanide poses a big threat to living organisms. It is potential contaminant most especially in water bodies. Heavy metals effluence is a serious threat to our environment as well, because it poses life threats to living organisms in aquatic, terrestrial and air habitats [2]. They are not degradable metabolically but build up in living tissue resulting to health problems and death of organisms [3].

Bacteria have developed resistance mechanism to tolerate the toxicity of heavy metal ions; they include efflux of metal ions outside the cell, amassing and complexation of metal ions within the cell and attenuation of heavy metal ions to a less noxious condition [4]. Generally a lot of bacterial species poses genes that manage resistance to particular toxic metals; this resistance is resolute by extra chromosomal DNA molecules for toxic metal ions including Co, Cd, Ni, Hg, Pb, Ag, Zn, Te etc [5]. This research is planned to assess the tolerance of locally isolated *Serratia marcescens* strain AQ07 to heavy metals in the degradation of potassium cyanide.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals utilized for the research are obtained from chemical agents. Heavy metals, Phosphate buffer, nutrient agar and bactor-agar media were supplied by Merck KGaA, Germany. Potassium cyanide (KCN) was acquired from R&M Chemicals; Essex, U.K. ɣ-Picoline (4-methyl pyridine) was purchased from ACROS Organics. Chloramine T sodium salt was supplied by Fisher Scientific, U.K. Sigma Aldrich CO Barbituric acid was used in this research.

Analytical methods

Optical Density 600nm was used for measurement of bacterial growth. Modified ɣ - picoline and barbituric acid method was used for the cyanide assay [6]. A phosphate buffer at pH 7.0 at

100 mM was used as the buffering species. ɤ - picoline and barbituric acid reagent was prepared by dissolving 3 g of barbituric acid was placed in a 50 ml conical flask containing 20 ml of deionised water. Then, 15 ml of ɤ - picoline (4-methyl pyridine) was added to the mixture while constantly stirring with magnetic stirrer. Concentrated hydrochloric acid (d=1.18) was added to the mixture and stirring was continuous. After the cooling down, the mixture was topped up to 50 ml with deionised water and stirred properly. This reagent is light yellow in colour.

In a dried centrifuge tube, 10 ml of diluted sample of cyanide solution with approximately less than 5 µg CN^- was placed. Then 5 ml of the phosphate buffer above was added and mixed thoroughly. 0.25 ml of 1% w/v chloramines T trihydrate solution was added. The mixture was stirred by vortexing. After 1–2minutes incubation at room temperature, 3 ml of ɤ-picoline and barbituric acid reagent was added to the mixture and it was incubated at 25 °C for 5 minutes. Absorbance was read at 605 nm against a reagent blank.

Preparation of buffer medium

Buffer medium was prepared with the following composition in 1 litre of deionised water: KH_2PO_4 – 7.2 g, K_2HPO_4 – 3.5 g, 10 ml of trace salts ($MgCl_2.6H_2O$ – 180 mg/L, $FeSO_4.7H_20$ – 300 mg/L, $CaCl_2$ – 40 mg/L, $Co(NO_3)_2.6H_2O$ – 130 mg/L, $ZnSO_4$ – 40 mg/L, MoO_3-20 mg/L) and 0.5 g yeast extract. The pH is adjusted to 6.0. It was autoclaved at 121 °C for 15 minutes [7]. Sterilized glucose – 5 g/L separately autoclaved from the medium to avoid caramelization i.e. reddish coloration, was added to the medium and 200 ppm filter sterilized KCN was added.

Biodegradation studies

1 ml of investigation sample was collected by the use of a sterilized micropipette and placed in a 1.5 ml sterilized centrifuge tube. The test sample was centrifuged by Sigma 1-4 Sartorius centrifuge at 10,000 x g for 10 minutes. 100 µl of test sample was drawn using sterilized micropipette and conveyed into a 15 ml centrifuge tube containing 9.9 ml of deionised water to dilute the sample. This method of analysis can only analyze less than 0.5 µg CN/ml [6]. The test sample was stirred by the use of a vortex machine. 500 µl of diluted test sample was transferred to a sterilized 1.5 ml centrifuge tube. 250 µl of buffer solution (pH 5.2) was placed into the tube and 13 µl of 1% (w/v) chloramine T sodium salt solution was also added. The tube was tightened and mixed gently. It was allowed to stay at room temperature for 1-2 minutes. 150 µl of ɤ-picoline-barbituric acid reagent was added and mixed thoroughly. The mixture was incubated at 25 °C for 5 minutes. Absorbance was determined at 605 nm using Shimadzu U.V. Mini 1240, spectrophotometer against reagent blank. The test was determined against an established linear standard curve range of 0.1 to 1 mg/L KCN.

Preparation of resting cells

A Modified method reported by Maniyam et al. [8] was used in this research work. Broth medium was prepared containing; Nutrient Broth – 8 g/L, Glucose – 8 g/L. It was sterilized by autoclave for 15 minutes at 121 °C. The medium was allowed to stand at room temperature until it is cool enough for inoculation. 2% of 24 hours pre-cultured *Serratia marcescens* strain AQ07 was inoculated. Aeration was supplied via BB-800 aquarium air pump fixed with chrome tech MCE 0.45 µm syringe filter. It was incubated at room temperature for a period of 48 hours. The bacterial cells yield were harvested at early stationery phase by centrifuging at 10,000 x g, 4 °C for 10 minutes using Beckman Coulter Avanti J-26 XPI centrifuge. The cells were washed two fold by the use of 100 mM phosphate buffer and then re-suspended in the same 100 mM phosphate buffer solution. The optical density was regulated to the array of 0.9 -1.0. It was kept at 8 °C for advance studies.

Effect of heavy metals on biodegradation and growth of resting cells of *Serratia marcescens* strain AQ07

Buffer medium as explained above was placed in a screw cap schott bottle and 1 parts per million (ppm) of heavy metals were placed in each bottle. All experiments were conducted in triplicates. The heavy metals include Silver, arsenic, cadmium, cobalt, chromium, cupper, mercury, nickel, lead and zinc. 20% of resting cells of the bacteria were added. It was incubated in an incubator shaker at 150 revolutions per minute (rpm) for 72 hours. 1 ml of sample was withdrawn; biodegradation analysis was carried out as described in item 2.4. Optical density ($O.D_{600}$) was measured using Shimadzu U.V. Mini 1240, spectrophotometer against reagent blank. The concentration of mercury was adjusted in an array of 0.1 to 1 ppm being the most effective heavy metal, and both analyses were repeated to ascertain the level of its effect on the bacteria.

RESULTS AND DISCUSSIONS

Heavy metals were tested against *Serratia marcescens* strain AQ07, to ascertain the effect on its ability to degrade cyanide. The toxicity of heavy metals ranges in an array of highest toxic to the lowest toxic element. Mercury (Hg^{2+}) is considered to be highly toxic and metals such as lead (Pb^{2+}) and aluminium (Al^{3+}) are considered to be lowest in toxicity [9]. Some elements such as zinc in low concentrations are very essential for some cellular processes of bacteria while higher concentrations are cytotoxic to the bacteria. However other elements such as (Pb^{2+}, Hg^{2+}, Cd^{2+}, Cr^{2+} and Ag^{2+}) have no known benefit to bacteria and some are very toxic even at lower concentrations [10].

Strain AQ07 is very effective in remediation of cyanide despite the incorporation of heavy metals. Mercury became the most active heavy metal that has effect on the bacteria which has inhibitory effect on both growth and biodegradation (**Table** I). This could be due to its affinity for thiol groups which is stronger, compared to cadmium affinity to sulphide as reported [4]. It has the capability of binding to sulfhydryl groups of enzymes, thus deactivating essential cellular functions [11]. It was able to remove only 24.7% of 200 ppm potassium cyanide and the growth of the bacteria stands at 0.13 compared to the control and other heavy metals. Silver (Ag), arsenic (As), cobalt (Co) were able to degrade 87.7% and lead (Pb) 87% of potassium cyanide with bacterial growth of 0.65, 0.68, 0.76 and 0.67 respectively (**Fig.**1). This indicates that the metals have less inhibitory effect on the bacteria.

The control which has no heavy metal incorporated record cyanide degradation of 92% and bacterial growth of 0.74. Cobalt illustrates high bacterial growth slightly above the growth obtained in the control sample. Cadmium, chromium and nickel exhibited degradation of 86.5% with bacterial growth record of 0.59, 0.66 and 0.67, respectively. Copper and zinc shows degradation effect of 86.6% with bacterial growth rate of 0.65 (**Table** I).

Table I. Effect of 1ppm heavy metals on growth and biodegradation of 200 ppm KCN.

Heavy Metals	Degradation (%)	Bacterial Growth
Silver (Ag)	87.7	0.65
Arsenic (As)	87.7	0.68
Cadmium (Cd)	86.5	0.59
Cobalt (Co)	87.7	0.76
Chromium (Cr)	86.5	0.66
Copper (Cu)	86.6	0.65
Mercury (Hg)	24.7	0.13
Nickel (Ni)	86.5	0.67
Lead (Pb)	87	0.67
Zinc (Zn)	86.6	0.65
Control	92	0.74

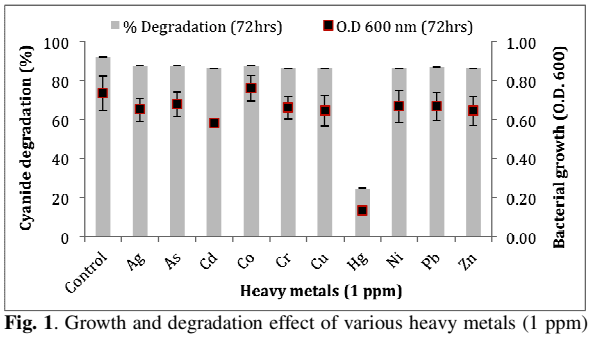

Fig. 1. Growth and degradation effect of various heavy metals (1 ppm) on the degradation of 200 ppm KCN by *Serratia marcescens* strain AQ07 in a period of 72 hours. Data represent mean ± STDEV, n = 3.

The ability to stand the toxicity of heavy metals and degrade cyanide is attributed to microbial detoxification mechanisms developed by the microorganisms that are capable of resisting toxicity such as exopolysacharides binding with bacterial cell envelopes, metal efflux, metal reduction, and so on. These materials are occasionally encoded in plasmid genes assisting the transport of toxic metal resistance from one cell to another [12]. The survival of microorganisms in polluted environment depends on inherent structural and biochemical properties, physiological and environmental adaptations of metal speciation [13]. Bacteria exhibits diverse resistance mechanisms in reaction to heavy metals, these mechanisms may be programmed by chromosomal genes, however most usual loci granting resistance are situated in the plasmids [14]. These mechanisms can be employed for degradation of heavy metals from polluted environment. Further research (**Fig.** 2) was conducted on mercury effect in order to identify the concentration that is most effective in exertion of its inhibitory effect on the bacteria.

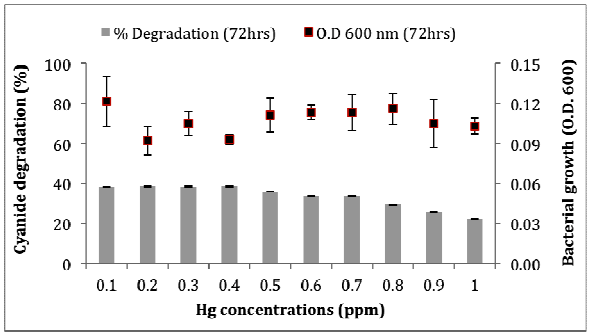

Fig. 2. Growth and degradation effect of mercury on the degradation of 200 ppm KCN by *Serratia marcescens* strain AQ07 in a period of 72 hours. Data represent mean ± STDEV, n = 3

Mercury was utilized in an array of concentration of 0.1 to 1.0 ppm to enable identification of less and more effective concentration on the bacteria. The metal seems to have a very serious effect on the strain because it exhibit poor degradation and growth ability even at the lowest concentration of 0.1 ppm

CONCLUSION

This research work illustrates the ability of *Serratia marcescens* strain AQ07 to stand the toxicity of heavy metals in the degradation of highly toxic potassium cyanide which makes it good bacteria to be used for the remediation of cyanide since it has the ability to stand the toxicity of various heavy metals. Though mercury proves to be very effective in the inhibition of this bacterial activity, all other heavy metals tested tends to be less effective. Mercury has been reported to be the most toxic heavy metal and many bacteria have less capability to stand its toxicity.

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