Growth Responses of Chromium (vi) Tolerant Bacteria to Different Concentrations of Chromium

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Abstract--Chromium (vi) – tolerant bacterial strains, namely, Bacillus sp., Pseudomonas sp., Escherichia coli and Staphylococcus sp. were isolated from the oxidation ditch of a sewage treatment plant. All the isolates tolerated high concentrations of up to 500 μg/ml chromium (vi) in nutrient broth. The growth responses of the bacterial isolates to different concentrations of chromium (vi) were carried out in this study. The responses of the bacteria were dependent on time of incubation and chromium (vi) concentration. The analysis of the results showed that there was significant difference (P < 0.05) in the growth of the isolates at different concentrations of hexavalent chromium. As the concentration of chromium increased, the growth of the bacterial isolates decreased. The growth of the isolates was slightly inhibited at hexavalent chromium concentration of 200 μg/ml and highly inhibited at 500 μg/ml when compared to other lower concentrations such as 50, 100 and 150 μg/ml. The present study revealed the capacity of the bacterial isolates to grow at different concentrations of Cr (vi) and such bacteria can be used to remove Cr (vi) from the environment.

Index Term--Chromium, tolerance, bacteria, remediation, growth response

I. INTRODUCTION
Chromium (Cr) compounds have widespread industrial uses in steel production, wood preservation, leather tanning, metal corrosion inhibition, paints and pigments, metal plating and other applications [1]. Hexavalent chromium generated from several of these industrial processes is discharged into the environment and is toxic and mutagenic to most organisms [2]. Chromium exists both in hexavalent and trivalent forms. However, hexavalent form is very toxic, carcinogenic and mutagenic both in humans and animals whereas trivalent form is less toxic, less soluble and thus a lesser problem.

Chrome plating, the deposition of metallic chromium, imparts a refractory nature to materials rendering the resistant to microbial attack and flexible over extended periods of time. More than 170,000 tonnes of Cr wastes are discharged to the environment annually as a consequence of industrial and manufacturing activities [3].

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Despite the fact that heavy metals are acutely toxic to most microbes, there are metal tolerant bacteria. Long term exposure to metals favours proliferation of microbes that are tolerant to metals and this has been investigated by assaying habitats exposed to anthropogenic or natural metal contamination over extended period [4].

Several physical and chemical methods exist to remove heavy metals such as chromium from the environments. However, these methods are reported to be impractical due to the operational high cost and subsequent generation of solid waste which is difficult to treat. Research in recent years indicated that many microorganisms accumulate large concentrations of metals [5]. Bioreduction of Cr (vi) can occur directly as a result of microbial metabolism (enzymatic) or indirectly, mediated by bacterial metabolic products (such as H2S) [6].

Microbial tolerance to hexavalent chromium has practical importance because it can serve as a basis for selecting organism that can be used to detoxify chromium in the environment [7]. A number of chromium tolerant microorganisms have been reported including Pseudomonas species [8]. Microbacterium [9], Enterobacter spp [10], Escherichia coli [11], Bacillus spp [12] and several other bacterial isolates [13].

The objective of this study was to evaluate the growth responses of chromium (vi) tolerant bacteria isolated from a sewage treatment oxidation pond to different concentrations of chromium.

II. MATERIALS AND METHODS

Microorganisms and culture conditions
A strain of each of Staphylococcus sp., Bacillus sp., Pseudomonas sp. and Escherichia coli was used throughout this work. They were isolated in our laboratory from sewage water collected from the University of Nigeria sewage treatment oxidation pond as described by Ezaka and Anyanwu [14]. The isolates were maintained on nutrient agar slants at a refrigeration temperature of 4°C. Each seed culture was prepared by inoculating a loop of the stock culture into 20 ml of nutrient broth, within a 200-ml conical flask. The isolates were incubated overnight in nutrient broth after which the bacterial cells were harvested, washed and resuspended in distilled water. To ensure equal cell population of each of the bacterial strains, their optical density was adjusted to be the same using spectrophotometer at 600nm.

Growth responses of the isolates
An aliquot of 2.5 ml of inoculum was transferred to 250 ml Erlenmeyer flask containing 50 ml of nutrient broth amended with chromium (vi) to give final concentrations of 50, 100, 150, 200 and 500 μg/ml. All experiments were carried out in triplicates. The incubation was carried out on a
rotary shaker (Gallenkamp) at 180 rev/min and 30°C for 96 h. Culture samples were taken at 24 h intervals during the incubation period and analyzed for optical density at 600 nm with a spectrophotometer (Spec21D, Pec Medical, USA). Control experiments were set up in parallel at the same time with inoculated flasks without chromium amendment.

**Statistical Analysis**

The data obtained in this study were analyzed using two way analysis of variance. Statistically significant treatment differences were considered with $P < 0.05$.

**RESULTS**

Four chromium (vi) tolerant bacteria isolated from sewage water sample collected from an oxidation pond were used for the present study. The isolates were identified as *Staphylococcus* sp, *Bacillus* sp, *Pseudomonas* sp. and *Escherichia coli*. The growth responses of the isolates to different concentrations of hexavalent chromium at different time intervals were determined using a spectrophotometer (Spec21D, Pec Medical, USA) at 600nm. The ability of the four bacterial isolates to grow at different concentrations (50, 100, 150, 200, and 500 μg/ml) of Cr (vi) in nutrient broth was evaluated by measuring the optical density of the cell suspension at 600nm at 24 h intervals. The results of these studies are shown in figs 1 to 4.

The analysis of the results showed that there was significant difference ($P > 0.05$) in the growth of the isolates at different concentrations of hexavalent chromium. As the concentration of chromium increased, the growth of the bacterial isolates decreased. The growth of the isolates was slightly inhibited at hexavalent chromium concentration of 200 μg/ml and highly inhibited at 500 μg/ml when compared to other lower concentrations such as 50, 100 and 150 μg/ml.

In fig. 1, the growth of *Bacillus* sp. at different concentrations of Cr (vi) showed a decrease in growth with increase in the concentration of the metal. The growth of the isolate was inhibited at chromium concentration of 500 μg/ml.

In fig. 2, the growth of *Pseudomonas* sp. at different concentrations of hexavalent chromium showed increase with increase in incubation time and decreased with increase in the concentration of Cr (vi). There was an initial decrease and subsequent increase in the growth of the isolate at the concentration of 500 μg/ml. The growth of *Pseudomonas* sp. decreased between 0 - 24 h and increased between 48 and 96 h. There was significant difference ($P < 0.05$) in the growth of the isolate at different concentrations and time. However, there was no significant difference in the growth of the isolate at 150 and 200 μg/ml chromium concentrations between 0 and 48 h of incubation.

In fig. 3, the growth of *Escherichia coli* at different concentration of Cr (vi) was shown. The growth of the isolate increased with increase in the incubation time at chromium (vi) concentrations of 50 and 100 μg/ml. There was no significant difference ($P < 0.05$) in the growth of the bacteria at a concentration of 150 μg/ml at different times. The isolate showed the highest growth at chromium concentration of 50 μg/ml whereas the growth of the isolate was inhibited at the concentration 500 μg/ml.

The growth response of *Staphylococcus* sp. to different concentrations of Cr (vi) are shown in figure 4. The growth of the isolate increased with increase in incubation time at the concentrations of 50, 100, 150 and 200 μg/ml while at 500 μg/ml, the growth decreased. At 500 μg/ml, the growth of the *E. coli* deceased drastically between zero and 24 h and later increased slightly with increase in incubation time. There was no significant difference ($p < 0.05$) in the growth of *E. coli* at 100 and 150 μg/ml, respectively.

![Fig. 1. Growth response of *Bacillus* sp. to different concentrations of Cr(vi)](image-url)
Fig. 2. Growth response of *Pseudomonas* sp. to different concentrations of Cr (vi)

Fig. 3. Growth response of *Escherichia coli* to different concentrations of Cr (vi)

Fig. 4. Growth response of *Staphylococcus* sp. to different concentrations of Cr (vi).
DISCUSSION
The decrease in growth at this concentration (500 μg/ml) may be as a result of toxic effect of the metal at this concentration whereas the subsequent increase may be as a result of the ability of the isolates to secrete enzyme that enable them to withstand such concentration; there was significant different (P < 0.05) between the growth of the isolate at different concentration and time.

Presence of heavy metal tolerant bacteria in a given environment may be an indication that such environment may contain heavy metals. It is likely that such area fosters adaptation and selection for heavy metal resistant microorganism [15]. Isolation of bacteria from metal–polluted environment would represent an appropriate practice to select metal resistant strains that could be used for heavy metal removal and bioremediation purpose [16]. In this study, the growth response to chromium (vi), of chromium tolerant bacteria has been reported. The bacterial isolates responded to a wide range of Cr (vi) concentrations namely 50,100,150, 200 μg/ml and 500μg/ml of Cr(vi). It was reported [17] of bacteria resistant to up to 100 mg/l Cr(vi). The chromium(vi) resistance above 2500 mg/l has also been reported by Shakoori et al. [18]. It has been reported that long term exposure to metals imposes a selection pressure that favors the proliferation of microbes that tolerate metals [4]. There was an initial decrease and subsequent increase in the growth of the isolates at the of 500 μg/ml concentration of Cr (vi). The decrease in growth at 500 μg/ml concentration may as be a result of toxic effect of the metal at this concentration whereas the subsequent increase may be as a result of the ability of the isolates to secrete enzymes that enable them to withstand such concentration or the organisms become adapted to the metal.

The growth of the isolates was dependent on time. The analysis of the result showed a significant difference (P < 0.05) in the mean growth of the isolates at different times. It also showed a significant difference (P < 0.05) in the mean growth of the isolates at different concentrations. The highest growth recorded at a concentration of 50μg/mg may be as a result of less toxic effect of the metal. However, growth decreased as the concentration of the Cr (vi) increased. The greatest decrease was observed at the highest concentration of 500μg/ml. This poor growth rate at 500μg/ml may be as a result of the toxic effect of the high concentration of the metal. Evidently, high chrome concentration prevented multiplication of bacteria [19].

CONCLUSION
Chromium(vi) concentration can be an important environmental factor regulating remediation strategies for ecosystem polluted with hexavalent chromium. The native isolates which tolerate different concentrations of Cr (vi) will be highly effective in Cr (vi) bioremediation which is potentially less expensive than chemical method in treatment of environment contaminated with Cr (vi). The present study revealed the capacity of the bacterial isolates to grow at different concentrations of Cr (vi) and such bacteria can be used to remove Cr (vi) from the environment. Further research is, therefore, needed to study the resistance potential of the isolates and to evaluate their ability to detoxify hexavalent chromium in the environment.

REFERENCES