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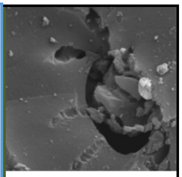

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Screening of Chromium-reducing Bacteria from Tannery Effluents

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ABSTRACT

Tannery effluent has remained one of the major sources of chromium pollution in the environment. Although conventional methods have been widely used, they are inefficient and costly. Bacterial remediation is one of the best alternatives being proposed. Therefore, the aim of this research was to isolate bacteria from tannery effluents and screen them for chromium-reduction potentials. Three different tannery effluents were collected and used for the isolation of chromium-reducing bacteria. The organisms were identified using morphological and biochemical characteristics and screened on 1% (v/v) Cr (VI). The results revealed the presence of three bacterial species, namely: *Bacillus subtilis, Escherichia coli* and *Pseudomonas* sp. The screening results revealed that out of the three bacterial isolates, *Bacillus subtilis* had the highest reduction potential (86.23%), while equal reduction capacity was recorded in both *E. coli* and *Pseudomonas* sp. (84.03%). Therefore, these three isolates can be used as a consortium to improve biological remediation of Cr (VI) effluents.

INTRODUCTION

Heavy metals pollution as a result anthropogenic activities has continued to be a source of concern globally [1]. The toxic heavy metals include cadmium, arsenic, mercury, lead and chromium, all of which are carcinogenic and present a potential threat to the environment even in small quantity (0.1-2 mg/L) [2,3]. Moreover, many reports have indicated that out of the listed toxic heavy metals, chromium has been considered one of the most toxic, especially chromium (VI). Chromium exits in different oxidation states such as Cr (III) and Cr (VI), however, Cr (VI) is considered the major pollutants which penetrate human body through oral, skin or nose [4]. Chromium pollution affects humans, animals, soil fertility, source of drinking water and causing adverse effects to the environment [2]. The major sources for chromium contamination include natural and anthropogenic sources such as tannery industry [5]. Among all the sources, contamination from tannery waste water is more pronounced as it contains about 2000-5000 mg/L Cr [6]. Tannery industry effluents not only contains chromium, but also toxic chlorides and sulphide, both of which are inhibitory to microbial growth, thus affecting biological treatment of water, amounting to low-quality drinking water supply [7]. Thus, treatment of chromium contaminated water becomes necessary.

Conventional treatment such as adsorption, membrane filtration precipitation and ion exchange were widely employed. However, many reports have indicated their major draw backs [8]. For example, precipitation method is expensive and generate a lot of effluents and sludge which cause secondary disposal problems [5,9]. However, bioremediation, being green and cost-effective, has been recently being considered as the best alternatives [10]. Several bacterial agents such as *Bacillus stratosphericus* and many members of *Actinobacteria*, *Proteobacteria* and *Firmicutes* have been used with great success for the remediation of chromium effluents [11]. Recent report has shown that *Bacillus subtilis* P13 was able to withstand 350 mg/L and 35 mg/L of chromium (III) and (VI), respectively, indicating high remediation potential [12].

Other bacteria such as *Leucobacter* sp. G161 can tolerate as high as 1000ppm of chromium and can reduce 400 ppm Cr (VI) to Cr (III) within four days of incubation [13]. Bacteria usually reduce chromium from Cr (VI) to a less toxic form, i.e., Cr (III), thus, making them best candidates for biological remediation [14]. Nevertheless, depending on the bacterial type, metal sequestration capacity differs among bacterial community due to the presence or absence of genes encoding reduction enzymes [14]. Therefore, search for more chromium reducing-bacteria in northern Nigeria, especially Bauchi state, where such published literature is limited, is ardently needed. Thus, the aim of this research was to isolate bacteria from tannery effluents and screen them for chromium-reduction potentials.

MATERIALS AND METHODS

Sample collection

Wastewater samples (150ml) from three different tannery industries: Gidan Alhaji Aminu Katagun L G A, Tannery station, Chelide, Kirfi Local Government Area (L. G.A) and Larrabee Enterprise located at Sharada Industrial estate, Gwale L.G.A, Kano State, Nigeria. The collected samples were brought to the microbiology laboratory, Bauchi State University Gadau for further processing.

Enumeration Determination of total chromium-reducing bacterial count

The chromium reducing bacteria were grown using enrichment culture technique. Five (5 ml) of the Tannery wastewater samples was transferred into 150 ml Erlenmeyer flasks containing 50 ml of nutrient broth (NA) amended with Cr (VI) (2%) and incubated at room temperature on an orbital shaker (150 rpm) for 5 days [15]. Following incubation period, 1 ml of enriched culture was serially diluted and inoculated onto sterilized nutrient agar using pour plate method. The plates were incubated at room temperature for 24 h and the colonies formed were enumerated and expressed as CFU/ml.
The colonies formed were further subculured and identified using colonial morphology, gram reaction and biochemical tests [16].

Screening of chromium-reducing bacteria

To test for chromium reducing potentials, the isolates were inoculated into 150 ml Erlenmeyer flasks each supplemented with 1% (v/v) Cr (VI) and 50 mL NB. The flasks were further incubated at room temperature with constant agitation (150 rpm) for 7 days. Cell growth was measured as optical density at 600 nm (OD_{600}) and was used as a parameter for screening the chromium reducing potential of the isolates using spectrophotometer [13].

RESULTS AND DISCUSSION

Chromium-reducing bacteria are mostly found in soil and water contaminated with chromium. In this study, effluents from three different tannery industries including Gidan Alhaji Aminu (A), Chelide (B) and Larrabee Enterprise (C) were collected and analyzed for bacteriological quality. The results revealed that the highest count was recorded in location C, followed by B and the least was recorded at location A (**Fig. 1**). The high increase in bacterial count in location C might be due to the increase disposal of organic matter along that area. (15) have examined the bacteriological quality of chromium waste water and found that lower basin had higher *E. coli and Enterococcus* sp., due to fecal matter contamination, thus indicating adaptability and remediation capability. Many reports have indicated that chromium-reducing bacteria can be isolated not only in the contaminated water but also in the soil environment [17] enumerated total chromium-reducing bacteria from three different soil samples and reported that sample 1 had the highest bacterial diversity while the least was recorded in sample three.

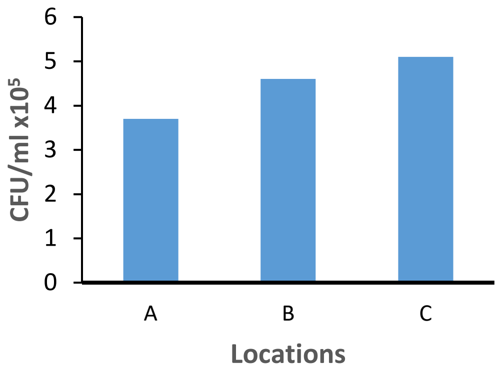

Fig. 1. Bacteriological quality of Tannery wastewater from three locations**.** Key: A= Chelide Kirfi L.G.A ; B= Gidan Alhaji Aminu Katagum L.GA; and C= Larrabee Enterprise, Gwale L.G.A.

In this study, three bacteria: *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas* sp. were isolated based on their morphological and biochemical characteristics. A wide range of bacteria such as *Enterococcus* sp., *E. coli* [15]*, Bacillus subtilis* P13 [12], *Leucobacter* sp. [13] were isolated from chromium contaminated water. Bacteria from these contaminated water are often resistant to chromium toxicity and can therefore be used for its remediation [18]. Tannery effluent being the main source of chromium contamination is widely used for screening of chromium-reducing organisms. Based on this, three bacterial isolates were screened for their potential to reduce Cr (VI). Out of the 3 isolates tested, *B. subtilis* had the highest reduction potentials (86.23%), while both *E. coli* and *Pseudomonas* sp. have the same reduction capacities (84.3%) after 7 days of incubation (**Fig. 2-4**). *Bacillus* sp. has been successfully used for the bioremediation of Cr (VI) effluents. Our result is in agreement with Zahoor's finding [19] who found that after 7 days of incubation, 86% of Cr (VI) was reduced by *Bacillus* sp. JDM-2-1 [20] isolated *Bacillus* sp. from RAMKY wastewater treatment plants, India and tested it ability to reduce Cr (VI). It was found that the isolate reduced 95% of 40 mg/L of Cr (VI) in 24 h, indicating it higher remediation potential. A newly identified *Bacillus stratosphericus* has recently shown to have higher Cr (VI) potential [11], suggesting superiority of *Bacillus* sp. in chromium reduction. In a different study, [17] isolated and screened 6 bacterial isolates for chromium reduction capacity. The results showed that *Bacillus odysseyi* YH2 was the best candidate and had reduced 23.5% of 350 mg/L Cr (VI).

In addition, [21] Screened 28 bacterial isolates from tannery wastewater for their Cr (VI) reduction potentials and contrary to our findings, concluded that *Klebsiella s*p. SH-1 was the best. The isolate was able to reduce 72% of Cr (VI) in a tannery wastewater and 95% reduction recorded in Luria-Bertani broth after 3 days of incubation. [22] isolated 5 bacterial isolates (*Serratia marcescens MKPF12, Klebsiella variicola MKPF8, Acinetobacter gerneri* MKPF7, *Klebsiella pneumonia MKPF5, Kosakonia cowanii MKPF2*) from tannery wastewater and screened them for Cr (VI) reduction.

Out of the 5 isolates, *Acinetobacter gerneri* MKPF7 had the highest potential. Reduction potentials of most isolate is limited due to toxicity of Cr (VI), however, their potentials could be improved by forming a microbial consortia or biostimulation.
Addition of carbon sources were reported to improve reduction of Cr (VI) [23] employed glucose as a substrate to improve Cr (VI) reduction over wide range of concentrations (10-100mg/L Cr^{+6}) by *Serratia* sp. The result revealed that 100% reduction was achieved in all the concentration except 100 mg/L, and was also completely reduced when high carbon iron filings was added to the media.

Pseudomonas sp. has been one of the best candidates employed in bioremediation of used engine oil, diesel, crude oil and other petroleum fractions [24–26]. Thus, *Pseudomonas* sp. can be used for remediation of chromium contaminated environment [27,28]. [29] tested bioreduction capacity of *P. aeruginosa* in the presence of goethite over a wide range of environmental factors and carbon sources. The findings showed that *P. aeruginosa* reduced 54% of Cr (VI). In a similar investigation, *P. aeruginosa* Rb-1 and *Ochrobactrum intermedium* Rb-2 Cr (VI) potentials were tested based on the physiological and biochemical responses. The results showed that *Ochrobactrum intermedium* Rb-2 was the best candidate [30].

Moreover, concentration of Cr (VI) has been reported as one of the factors influencing bioremediation efficiency [31] tested the efficiency of a bacterial consortium (*Comamonas* sp., *Delftia* sp., *Clostridioides* sp., *Alicycliphilus* sp., *Acidovorax* sp. and *Bacillus* sp.) over different concentration of Cr (VI) i.e., 269 mg/L, 355 mg/L and 435 mg/L in 93.5 h. The results revealed that 100%, 85% and 61% reductions were observed in 269 mg/L, 355 mg/L and 435 mg/L Cr (VI) concentrations, respectively. This indicates the influence of Cr (VI) concentration and the capacity of a bacterial consortium to overcome such problem.
E. coli has been widely used for chromium reduction as reported by various literatures (32–35]. Although reduction percentage of *E.coli* is relatively low in this study (**Fig. 3**), *E. coli* FACU was reported to efficiently reduced 100 mg/L Cr (VI) to Cr (III) [33]. The application of genetic engineering for improving Cr (VI) reduction is recently gaining wide acceptance. *E. coli* strain eChrAB had been used efficiently to improve Cr (VI) reduction using genetic engineering technique [36].

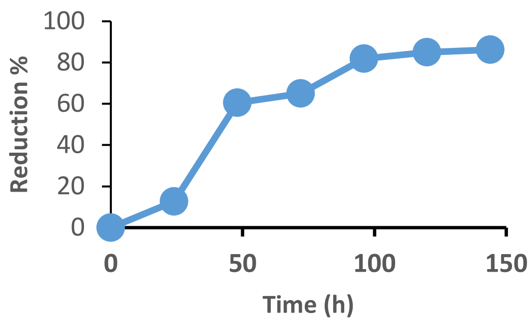

Fig. 2. Reduction potentials of *B. subtilis* on 1% chromium (VI).

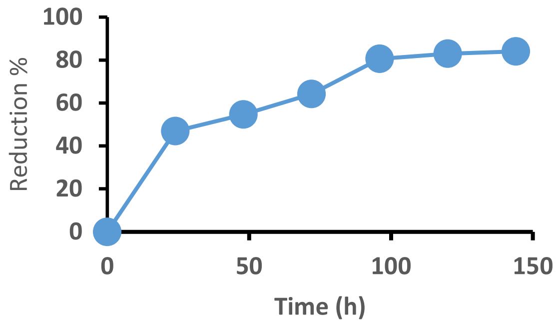

Fig. 3. Reduction potentials of *E. coli* on 1% chromium (VI).

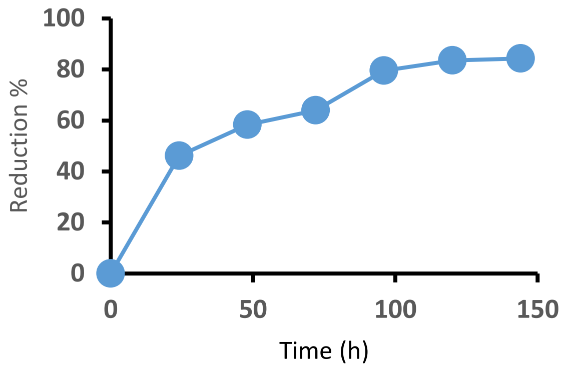

Fig. 4. Reduction potentials of *Pseudomonas* sp. on 1% chromium (VI)

CONCLUSION

Chromium-reducing bacteria are abundant in nature and can be isolated from myriads sources such as soil water and Cr (VI) contaminated sludge. In this study, three bacteria: *B. subtilis, E. coli* and *Pseudomonas* sp. were isolated from effluents generated by 3 tannery processing industries in northern Nigeria and screened for Cr (VI) reducing potentials. Among the three isolates tested, *B. subtilis* was proven to be the best with 86.23% reduction. This indicates potentials of *B. subtilis* in remediation of chromium polluted environment. In addition, since Cr (VI) is highly toxic and lethal to many microbes, these can be overcome by forming a microbial consortium, addition of carbon and nitrogen sources (biostimulation), and genetically engineering the microbes. Many reports have shown that each of these three suggestions were relatively effective. Thus, future research should focus on combining these three approaches concomitantly for the improvement of Cr (VI) reduction.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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