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Original Research Article

Isolation and Molecular Characterization of Indigenous Bacteria from Tannery Effluent and Their Potential for Bioremediation of Heavy Metals

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ABSTRACT
Tannery effluent refers to as wastewater from the process of converting skin and hides into leather. This study was aimed at the Isolation and molecular characterization of indigenous bacteria from Tannery effluent sites including Unguwar Karaye A (UKA), Unguwar Karaye B (UKB), Unguwar Rogo A (URA) and Unguwar Rogo B (URB). Effluent Sample were collected in sterile bottles and serially diluted (10^{-1} to 10^{-7}) before being inoculated on to nutrient agar using spread or pour plate techniques., after incubation at 30c° to 37°c for 24 to 48hours, distinct colonies were isolated, and identified through Gram staining, biochemical test and molecular methods such 16S rRNA sequencing. The physicochemical analysis showed acidic pH ranging from 5.25±0.19 to 6.87±0.29 with temperatures between 34°c±0.82 and 37°c±1.25. Total viable bacterial count ranges from 1.32×10^{7} to 1.75×10^{7}cfu/g. Twelve (12) bacterial species were identified, with *Bacillus subtilis* being the most predominant (21.1%), followed by *Exiguobacterium profundum* (10.5%), *Bacillus pumilus* (10.5%), *Pseudomonas aeruginosa* (10.5%), *Staphylococcus aureus* (10.5%), *while Bacillus licheniformis* (5.3%), *Pseudomonas fluorescence* (5.3%), *Pseudomonas putida* (5.3%), *Enterobacter cloacae* (5.3%), *Enterobacter aerogenes* (5.3%), *Escherichia coli* (5.3%) and *Acinetobacter baumanii* (5.3%) were the least prevalent. Chromium concentration from 400mg/L was reduced by bacteria *Exiguobacterium profundum* to 0.523 ±0.004 and 0.175±0.02 within 48hours and 96hours, outperforming *Pseudomonas aeruginosa*. Molecular identification confirmed the most efficient isolate as *Exiguobacterium profundum* strain 10C. This study highlights its strong potential for chromium bioremediation in tannery effluents.

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INTRODUCTION

The leather tanning industry is one of the most significant industrial activities worldwide, producing a wide range of leather goods used in fashion, automotive, and various other applications. However, the tanning process generates large amounts of highly contaminated wastewater, known as tannery effluent (Abdullahi *et al*., 2025). This effluent contains a complex mixture of pollutants, including heavy metals, organic chemicals, and dyes, which, when improperly treated, can have severe ecological and health impacts. Heavy metals, in particular, are major concern due to their toxicity, persistence in the environment, and potential to accumulate in living organisms, leading to detrimental effects on aquatic ecosystems and human health (Kolya and Khang, 2024).

Tannery effluent refers to as wastewater from the process of converting skin and hides into leather. The process of tanning requires a large volume of water, which is used to either cleanse the hides and skins or as an interaction medium between the hides and skin. During the tanning process, a large volume of effluents is discharged into the surrounding soil as well as a water source (Singh *et al.,* 2023).These effluents may contain a variety of chemicals such as sodium sulfate, chromium sulfate, and non-ionic wetting agents that are used in the tanning process and may accumulate in the immediate environment of the tannery (Nur-E-Alam *et al*., 2020).

A tannery is one of the important industries causing water pollution and there are more than 6000tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins. It was reported that the total amount of waste produced per slaughtered animal is approximately 35% of its weight and also, for every 1000kg of carcass weight, a slaughtered beef produces 5.5kgof manure (excluding rumen contents or stock yard manure) and 100kg of paunch manure undigested food (Hira *et al.,* 2022).Tannery effluents typically contain not only chromium but also other harmful metals, including lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), and zinc (Zn). These metals can pose a significant environmental threat, as they accumulate in soils, water bodies, and organisms, thus threatening biodiversity and posing a risk to human health. For example, Cr (VI) is highly soluble in water and can be easily absorbed by plants and animals, causing chronic toxicity, while lead and cadmium are known to damage kidneys and neurological functions (Sharma *et al*, 2021).

Conventional treatment methods for heavy metal removal, such as chemical precipitation, ion exchange, and membrane filtration, often have limitations, including high operational costs, the generation of secondary pollutants, and inefficiency in treating complex waste water mixtures (Saleh*et al.,* 2022). Traditional methods for treating tannery effluent include physical and chemical processes such as coagulation, flocculation, chemical precipitation, and ion exchange. While these methods can be effective in removing pollutants, they often involve high operational costs, large amounts of chemicals, and the generation of secondary wastes that require further disposal. Moreover, these conventional treatments are not always successful in removing the complex mixture of contaminants present in tannery effluent, especially heavy metals (Bhardwaj *et al.,* 2023).

Chromium, particularly hexavalent chromium (Cr (VI)), is widely used in the tanning process to enhance the durability and flexibility of leather. However, Cr (VI) is highly toxic, carcinogenic, and mobile in the environment. In addition to chromium, tannery effluents contain other harmful substances, including sulfides, phenols, and organic compounds, all of which contribute to environmental pollution. When improperly managed, these pollutants can severely affect aquatic ecosystems, ground water quality, and human health, creating an urgent need for efficient and sustainable effluent treatment strategies (Ahmed *et al.,* 2021).

Bioremediation is the process by which living organisms degrade or transform pollutants, has emerged as a promising approach for treating contaminated environments. Among the various types of bioremediation, microbial bioremediation, which uses microorganisms like bacteria, fungi, and algae, has gained significant attention due to its efficiency, cost effectiveness, and minimal environmental impact (Bala *et al.,* 2022). The use of indigenous bacteria which are naturally adapted to their local environment, presents several advantages over non-native species for bioremediation purposes. Indigenous bacteria possess intrinsic mechanisms that allow them to survive and thrive in the polluted environments from which they are isolated (Singh and Kostova, 2024). These bacteria are more likely to be effective in treating local contaminants, as they have evolved specific metabolic pathways and tolerance mechanisms to handle the pollutants present in their environment. In the context of tannery effluent, indigenous bacteria are well suited for the bioremediation of heavy metals, as they are already adapted to the harsh conditions, including the high concentrations of toxic substances that often characterize tannery waste water (Roy *et al.,* 2024).

The microbial diversity in tannery effluent is vast, and many different bacterial species have been reported to exhibit tolerance to heavy metals. Some bacteria are capable of absorbing heavy metals from the surrounding environment through biosorption, in which the metals bind to the cell surface, while others are capable of bioaccumulating metals inside their cells. Some bacterial species can even transform toxic metals into less harmful forms (Jayam and Chokkalingam, 2024). For example, certain bacteria are able to reduce hexavalent chromium (Cr(VI)) to trivalent chromium(Cr(III)), a less toxic and less soluble form, which

can then be more easily immobilized or removed from the environment. The identification and characterization of such bacteria are crucial for developing effective bioremediation strategies that can be applied to the treatment of tannery effluents (Wu *et al.,* 2024).

The potential of indigenous bacteria for bioremediation of heavy metals in tannery effluent remains an area of active research. Several studies have demonstrated the ability of bacteria isolated from contaminated environments to degrade or remove metals like chromium, cadmium, and lead. For example, bacteria such as *Pseudomonas putida, Bacilluscereus, and Serratiamarcescens* have been shown to exhibit significant metal tolerance and bioremediation capabilities (Monga *et al.,* 2022).

The aim of this research was to uncover some novels microbial strains by isolation and molecularly characterize capable of bioremediation of heavy metals.

MATERIALS AND METHODS

Study Area

The study was conducted in Sokoto Metropolis, Northwestern Nigeria (latitudes 13°03'N–13°15'N and longitudes 5°10'E–5°22'E), within the Sudan savanna ecological zone characterized by high temperatures and distinct wet and dry seasons (Ogwuche *et al.,* 2019). Sokoto state, being the seat of the caliphate, was for long having certain popular areas where residents have been practicing tanning process as their only source of income. Unguwar Karaye and Unguwar Rogo are one of the areas where local tannery activities are happening which are located within the Sokoto metropolis. During the process, large volumes of waste water (effluents) are being discharged into the soil environment leading to pollution (Ibrahim, 2014).

Collection of Samples

Tannery effluent sample were collected from Unguwar Karaye and Unguwar Rogo tannery as described by the method of Cheesbrough (2006) and American Public Health Association (1999) using clean sampling bottles which was labelled respectively. The sample (tannery effluent) was taken from the point in the facility premises where the effluent was thoroughly mixed and discharged before getting to the collection reservoir. After collection, the sample was transported to the Microbiology Research laboratory, Department of Microbiology, Usmanu Danfodiyo University Sokoto.

Physicochemical Analysis

Determination of pH and Electrical Conductivity (EC) of the Samples

The pH and Electrical Conductivity (EC) of the sample was determined according to Tijjani (2014) where an electronic method using water quality meter was employed. A calibrated pH meter was used to measure the pH of tannery effluent. For electrical conductivity, the (EC) electrode was calibrated using a standard buffer and the probe was inserted into tannery effluent and allowed for 30-60 seconds to maintain a steady reading. The reading displayed was record as micro Siemens per centimeter (µS/cm).

Determination of Temperature, Total Dissolved Solid and Total Suspended Solid

Temperature, total dissolved solid (TDS) and total suspended solid (TSS) was determined using water quality meter as described by Tijjani (2014) and the reading was recorded as mgL^{-1} respectively.

Determination of Dissolved Oxygen (DO) and Biological Oxygen Demand

Dissolved oxygen (DO) and Biological Oxygen Demand (BOD) was determined using the water quality meter as described by methods of Radojavik and Bashkin (1999). The electrode of the instrument was inserted into the effluent to determine the DO at point of collection. While BOD was obtained after the sample collection, diluted with sterile distilled water a hundred folds and allow for 5 days. The BOD was determined by inserting the electrode into the bottle and then subtracting the initial DO from the final value to obtain (BOD). The reading was recorded as milligrams per litre (mg/L).

Media Preparation

Twenty eight (28) gram of nutrient agar powder was weighed using weighing balance and placed in a conical flask and dissolved with 1liter of distilled water and heated with hot plate till it form a homogeneous solution. Then it was then autoclaved for 15 minutes at 121°C, allowed to cool to 45°C before dispensing into sterile petri dishes (Cheesbrough, 2006).

Heterotrophic Bacterial Counts

A series of sterile test tubes were labelled with appropriate dilution factors. 1ml of each sample wasintroducedinto 9 ml of sterile distilled water ($\times 10^1$). The tube was mixed thoroughly to ensure homogeneity. A volume of diluted solution was transferred (1 mL) from the first tube to the second tube ($\times 10^2$), until it reaches $\times 10^5$. Using spread plate method technique, 1 ml of the suspension from dilution of 10^{-5} was plated aseptically on a prepared nutrient agar (NA) and incubated at 37°C for 24hrs. The result was determined by multiplying the number of counts with the dilution used and expressed as colony forming units per gram (cfu/g) of samples. The colonies with distinct features were sub-cultured onto another nutrient agar to obtained pure culture of isolates (Agu, 2015).

Morphological and Biochemical Characterization of Bacterial Isolates

Gram Stain

Gram's staining was carried out as described by Didiugwu and Chukwura, (2023). Smear of the bacterial isolates wasmake on a clean grease-free glass slide using a drop of water. The smear was allowed to air dry and then passed over flame in order to be fixed. After fixing, the smear was covered with primary dyes (crystal violet) for one minutes then washed off with water. The slide was covered with Lugol's iodine for 60secs and washed off. Decolourizer was used to rapidly decolorize the smear and washed with water. Then, slide was covered with safranin for 60secs and washed off. The slide was allowed to air dry, and examined using oil immersion objective lens (×100) of the microscope.

Spore Staining

A smear was made on a slide and heat fixed. Malachites green (5%) solution was apply and heat until steam rises and allow to cool, and wash gently with cool water. The smear was counterstained with 0.5% safranin for 30seconds and wash with water. The slide was blot dry and examined under oil immersion objective lens for the presence of spores (Didiugwu and Chukwura, 2023).

Catalase Test

A culture of 18-24 hours was collected and placed in a clean slide. One drop of hydrogen peroxide solution was place on the culture organism in the microscopic slide. Air bubbles was observed in the slide and this represents the positive result (Princewill *et al.,* 2024).

Starch Hydrolysis Test

Starch is a complex carbohydrate that can be hydrolyzed by certain enzymes, such as amylase, produced by some bacteria. The starch-containing agar plate was inoculated with the test organism and incubated at 37°C for 24hrs. After incubation, the plate was flooded with lugol's iodine solution. A positive result will show a clear zone around the bacterial growth, while negative result will show no clear zone (Umar *et al.,* 2024).

Citrate Utilization Test

To a sterile Simon's citrate medium, a loopful of 24hours old isolated was inoculated aseptically and incubated at 37°c for 24hours. The media was examined daily for turbidity for period of 3 days. Turbidity indicated citrate utilization (Sabo *et al.,* 2024).

Methyl Red – Voges Proskauer Test

Five milliliter (5ml) of MR-VP broth was inoculated with the test organism and incubated for 24hours at 37^{0}C. Then one (1) ml of the broth was transferred to a small serological tube. Two (2) drops of methyl red was added to this quantity. A red colour signifies a positive test while yellow signifies a negative test. Five (5) drops of 40% potassium hydroxide (KOH) followed by fifteen (15) drops of 5% naphtol in ethanol was loosened and placed in a slanting position. The development of red colour starting from the liquid-air interface within an hour will indicate a VP positive test. No change in colour will indicate a VP negative test (Oyeleke and Manga, 2008).

Triple Sugar Ions Test

This medium contains three sugar namely glucose, sucrose and lactose. Some organisms can ferment all three sugars present and produce acid which changes the colour of the indicator from red to yellow. The production of H_2S can be detected by the presence of a black colour in the media along the stabbed line. Gas production was detected by the presence of gas bubbles or crack on the agar in the test tube or complete disruption of the medium. The test organism was inoculated onto the TSI slant by stabbing the butt down to the bottom of the slant. After an incubation period of 24-48 hours at 37°C; acid butt-alkaline slant (yellow butt, red slant) will indicate glucose has been fermented but not sucrose or lactose. Acid butt-acid slant (yellow but, yellow slant) indicates lactose and/or sucrose has been fermented. Alkaline butt-alkaline slant (red butt, red slant) will imply that neither glucose nor sucrose or lactose has been fermented (Hafezi *et al.,* 2024).

Urease Test

Colony from the stock culture was sub cultured into nutrient agar to obtain a fresh culture. Heavy inoculums was fetched from the nutrient agar using sterile wire loop and streaked on the slant surface of the urea medium. It was incubated for 24hrs at 37^{0}C. The development of a pink/red signifies urease positive; if colour remains unchanged (yellow/orange) it signifies negative (Cheesbrough, 2006).

Indole Test

The isolates were grown for 48hrs in test tube containing 5ml peptone water, 0.5ml of Kovacs reagent was added and shaken gently. The presence of a red or pink layer indicates the presence of indole which also indicate positive for indole, while absence of red colour indicated negative (Cheesbrough, 2006).

Chromium Tolerance Assay

Investigation into bacterial growth in the presence of different concentrations of chromium to screen for chromium tolerance were performed from potassium dichromate ($K_2Cr_2O_7$) compound at 100, 200, 300 and 400mg/l and tolerance were checked for 48 hours and 96 hours respectively. Bacterial growth were determined by

taking optical density using spectrophotometer at 600nm (Kabir *et al.*, 2018).

Molecular Characterization of Bacterial Isolates

DNA Extraction

DNA was extracted usisng the protocol stated by Balakrishnan *et al.* (2022). Briefly, single colonies grown on medium will be transferred to 1.5ml of liquid medium and culture were grown on a shaker for 48hours at 28°c. After this period, culture was centrifuged at 4600g for 5minutes. The resulting pellet will be resuspended in 520µL of TE buffer (10mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3µL of Proteinase K (20mg/ml) will then be added. The mixture was Incubated for 1hour at 37°c, 100uL of 5M Nacl and 80µL of a 10% CTAB solution in 0.7M Nacl will be added and vortexed. The suspension will Incubated for 10min at 65°c and keep on ice for 15min and centrifugation at 7200g for 20min. The aqueous phase will then transfer to a new tube and isopropanol (1:0.6) will add and DNA precipitated at -20oc for 16 hours. DNA was collected by centrifugation at 13000g for 10min, washing with 500µL of 70% ethanol, air dried at room temperature for approximately three hours and finally dissolve in 50µL of TE buffer.

16S rRNA Gene PCR Amplification

DNA amplification via PCR was carried out to selectively replicate target DNA sequences. Initially, genomic DNA was extracted from the sample and incorporated into a reaction mixture containing specific primers, deoxynucleotide triphosphates (dNTPs), reaction buffer, and Taq DNA polymerase. The amplification process began with an initial denaturation at 94–95°C for 2–5 minutes to separate the DNA strands. Subsequently, 25–35 thermal cycles were performed, each consisting of denaturation at 94–95°C for 30 seconds. Annealing at 50–65°C for 30 seconds (temperature depending on primer Tm). Extension at 72°C for approximately 1 minute per kilobase of target DNA. A final extension step at 72°C for 5–10 minutes was included to ensure complete synthesis of all DNA strands. The resulting amplicons will then visualized and analyzed using gel electrophoresis or subjected to downstream applications such as sequencing and phylogenetic analysis. Forward and reverse primers was used, each 18–25 nucleotides in length with melting temperatures typically ranging from 50–65°C.The forward primer annealed to the 3' end of the sense strand and extended in the 5' to 3' direction. The reverse primer annealed to the 3' end of the antisense strand, also extending 5' to 3'. Primers was carefully designed to avoid self-complementarity and secondary structure formation such as primer-dimers. The amplification of bacterial 16S rRNA genes used the following primer pair: Forward Primer: 5'-AGAGTTTGATCCTGGCTCAG-3'. Reverse Primer: 5'-GGTTACCTTGTTACGACTT-3' (Odeyemi *et al*., 2018).

PCR Product Purification

Isolates reducing heavy metal was purified by two procedures- Ammonium sulphate precipitation method and ZnCl2 precipitation method. By Ammonium sulphate precipitation method: it consists of four steps, ammonium sulphate fractionation, chilled acetone, hexane treatment, and silica gel column chromatography. By ZnCl2 precipitation method: 10 ml of the culture supernatants were concentrated by ZnCl2 to final concentration of 75mM. The precipitated material was dissolved in 10 ml Sodium phosphate buffer (pH 6.5), extracted twice with equal volumes of diethyl ether. Pooled organic phase were evaporated to dryness and pallets were dissolved in 100 µl of methanol. Further purification was achieved by preparative TLC (Odeyemi *et al*., 2018).

DNA Sequencing of 16S rRNA Gene Fragment

By using ABI DNA 3730 XL for sequencing sequencer (Applied Bio system), the 16S rRNA purified PCR product were submitted. Sequencing of the bacterial isolate's 16S rRNA gene was carried out in both directions. The bacterial species were determined with the obtained sequence which was searched for BLAST. The sequences were submitted to the NCBI Gen Bank after sequence matching percentages and accession numbers were obtained (Balakrishnan *et al.,* 2022).

Data Analysis

The data were presented in mean standard deviation and tables using Microsoft office excel spread sheet (Excel, 2010) and analyzed using Statistical Package for Social sciences (SPSS) version 20. LSD (least significant difference) test was used to identify significant differences among treatment means. P values < 0.05 were considered significant in all cases (Fiandini *et al.,* 2024).

RESULTS AND DISCUSSION

Physicochemical Characteristics of Tannery Effluent

Table 1 presents the physicochemical characteristics of the tannery effluents from Unguwar Karaye (A and B) and Unguwar Rogo (A and B) of Sokoto Metropolis. The mean pH of the effluent were recorded as 5.25, 5.60, 6.87 and 6.73 for URA, URB, UKA and UKB which happened to be acidic. The mean temperatures were also obtained as 36^0C, 37^0C, 35^0C, and 34^0C for URA, URB, UKA and UKB respectively. Electrical conductivity was also recorded as 651µs/cm, 652µs/cm, 660µs/cm and 662µs/cm respectively. Other parameters including dissolved oxygen were recorded as 8.1mg\l, 8.37mg\l, 9.27mg\l and 9.5mg\l for URA, URB, UKA and UKB. The Biological oxygen demand was also obtained as 21.2mg\l, 21.3mg\l, 31.1mg\l and 31.57mg\l for URA, URB, UKA and UKB respectively. The

mean Total dissolved solid of the effluent were recorded as 3500mg\l, 3505mg\l, 3550mg\l, 3528mg\l for URA, URB, UKA and UKB and Total suspended solid were also recorded as 970mg\l, 988mg\l, 896mg\l and 901mg\l URA, URB, UKA and UKB .

Heterotrophic Mean Bacterial Counts of the Tannery Effluent

Table 2 presents the bacterial counts of the tannery effluent samples obtained from Unguwar Karaye (A and B) and Unguwar Rogo (A and B) of Sokoto Metropolis, Sokoto State Nigeria. Sample A of Unguwar Karaye has the highest bacterial count of 1.75×10^7cfu/g while Unguwar Rogo A has the lowest bacterial count which is 1.32×10^7cfu/g.

Morphological and Biochemical Characterization of the Bacterial Isolates of the Tannery Effluent

Table 3 presents the morphological and biochemical properties of the bacteria isolated from the tannery effluent samples. The bacterial species identified were *Bacillus lichenformis, Bacillus subtilis, Exiguobacteriumprofundum, Bacillus pumilus, Pseudomonas aeruginosa, Pseudomonas, fluorescence, Pseudomonas putida, Staphylococcus aureus, Enterobacter cloacae, Enterobacteraerogenes, Escherichia coli* and *Acinetobacterbaumanii*.

Table 1: The Physicochemical Characteristics of Tannery Effluent Samples

Parameters	URA	URB	UKA	UKB
Colour	Brown	Brown	Brown	Brown
Odour	Foul Smell	Foul Smell	Foul Smell	Foul Smell
pH	5.25±0.19	5.60±0.05	6.87±0.29	6.73±0.58
Temperature (^{0}C)	36±1.63	37±1.25	35±1.63	34±0.82
EC (µs/cm)	651±1.25	652±2.45	660±6.23	662±3.09
DO (mg/l)	8.1±0.16	8.37±0.39	9.27±0.39	9.5±0.41
BOD (mg/l)	21.2±0.82	21.3±0.43	31.1±0.82	31.57±1.32
TDS (mg/l)	3,500±4.08	3,505±4.51	3550±6.38	3528±4.55
TSS (mg/l)	970±3.27	988±3.61	896±4.5	901±3.68

Keys: UKA = Unguwar Karaye Site A, UKB = Unguwar Karaye Site B, URA = Unguwar Rogo Site A, URB = Unguwar Rogo Site B, EC = Electrical Conductivity,DO = Dissolved Oxygen, BOD = Biological Oxygen Demand, TDS = Total Dissolved Solid, TSS = Total Suspended Solid.

Table 2: Heterotrophic Mean Bacterial Counts of Tannery Effluent

Samples	Plate Counts	Standard (Cfu/g)
UKA	175	1.75×10^7
UKB	152	1.52×10^7
URA	132	1.32×10^7
URB	162	1.62×10^7

Keys: UKA = Unguwar Karaye Site A, UKB = Unguwar Karaye Site B, URA = Unguwar Rogo Site A, URB = Unguwar Rogo Site B.

Table 3: Morphological and Biochemical identification of Bacterial Isolates

Samples	G	Shp	Sp	Gl	Su	La	Gs	HS	Ox	Ur	St	Ca	MR	VP	In	Ci	Organisms
UK1	+	Rod	+	+	+	-	-	-	+	-	+	+	-	+	-	+	*Bacillus subtilis*
UK2	+	Rod	+	+	+	-	-	-	+	-	+	+	+	-	-	-	*Bacillus pumilus*
UK3	+	Rod	+	+	+	-	-	-	+	+	+	+	+	+	-	+	*Exiguobacteriumprofundum,*
UK4	+	Rod	+	+	+	+	+	-	-	-	+	+	+	+	-	-	*Bacillus licheniformis*
UK5	-	Rod	-	-	-	-	-	-	+	+	+	+	-	-	-	+	*Pseudomonas aeruginosa*
UK6	-	Rod	-	-	-	-	-	-	+	+	+	+	-	+	-	+	*Pseudomonas fluorescence*
UR1	-	Rod	-	-	-	-	-	-	+	+	+	+	-	-	-	-	*Pseudomonas putida*
UR2	+	Coc	-	+	+	+	-	-	-	+	+	+	+	-	-	-	*Staphylococcus aureus*
UR3	-	Rod	-	+	+	+	+	-	-	-	-	+	-	+	-	+	*Enterobacteraerogenes*
UR4	-	Rod	-	+	+	+	+	-	-	-	-	+	+	-	+	-	*Escherichia coli*
UR5	-	Coc	-	+	-	-	-	-	-	-	-	+	-	-	-	+	*Acinetobacterbaumanii*
UR6	-	Rod	-	+	+	+	+	-	-	-	-	+	-	+	-	+	*Enterobacter cloacae*

Keys; G= Gram Reactions, UK = UnguwarKaraye, UR = UnguwarRogo, Coc = Cocci, Shp= Shapes, Sp= Spores, Gl=Glucose, Su=Sucrose, La=Lactose, Gs=Gas Production, HS= Hydrogen Sulphide, Ox=Oxidase, Ur=Urease St=Starch, Ca=Catalase, MR=Methyl Red, VP=Voges-proskaurer, In=Indole, Ci=Citrate.

Frequency of Occurrence of the Bacterial Isolates of Tannery Effluent

Table 4 presents the frequency of occurrence of the bacterial isolates. A total of twelve (12) bacterial species with varying frequency of occurrence were identified from the tannery water samples. *Bacillus subtilis* had the highest percentage frequency of occurrence (21.1%), which was found to be the most predominant species among the isolates that were isolated from the tannery water samples. Followed by *Exiguobacterium profundum* (10.5%), *Bacillus pumilus* (10.5%), *Pseudomonas aeruginosa* (10.5%), *Staphylococcus aureus* (10.5%), *while Bacillus licheniformis* (5.3%), *Pseudomonas fluorescence* (5.3%), *Pseudomonas putida* (5.3%), *Enterobacter cloacae* (5.3%), *Enterobacter aerogenes* (5.3%), *Escherichia coli* (5.3%) and *Acinetobacter baumanii* (5.3%) were the least prevalent.

Chromium Biosorption Assay by the Bacterial Species at 600nm Wavelength

Table 5 presents chromium biosorption assay at 600nm wavelength. Concentration of chromium (VI) heavy metal from $K_2Cr_2O_7$ compound at 100, 200, 300 and 400mg/l were tolerated for 48 and 96 hours. The reduction of wavelength was recorded as the duration progressed from 48 to 96hrs. *Exiguobacterium profundum* showed immense chromium biosorption potential among the isolates.

Table 4: Frequency of Occurrences of the Identified Bacterial Isolates

S/N	Identified Bacteria	Frequency of Occurrence	Percentage Frequency (%)
1	*Bacillus subtilis*	4	21.1
2	*Exiguobacteriumprofundum*	2	10.5
3	*Bacillus pumilus*	2	10.5
4	*Bacillus licheniformis*	1	5.30
5	*Pseudomonas aeruginosa*	2	10.5
6	*Pseudomonas fluorescence*	1	5.30
7	*Pseudomonas putida*	1	5.30
8	*Staphylococcus aureus*	2	10.5
9	*Enterobacter cloacae*	1	5.30
10	*Enterobacteraerogenes*	1	5.30
11	*Escherichia coli*	1	5.30
12	*Acinetobacterbaumanii*	1	5.30
	TOTAL	19	100

Table 5: Chromium Biosorption Assay by the Bacterial Species Isolated from Tannery Effluent at 600nm Wave Length

S/N	Species	Time	Concentrations			
			100mg/l	200mg/l	300mg/l	400mg/l
1	*Bacillus subtilis*	48hrs	0.248±0.003	0.306±0.003	0.488±0,002	0.580±0.003
		96hrs	0.196±0.003	0.216±0.001	0.296±0.002	0.482±0.002
2	*Bacillus pumilus*	48hrs	0.237±0.002	0.233±0.002	0.451±0.006	0.533±0.005
		96hrs	0.135±0.002	0.219±0.004	0.357±0.003	0.413±0.006
3	*Exiguobacteriumprofundum*	48hrs	0.237±0.002	0.380±0.001	0.387±0.001	0.523±0.004
		96hrs	0.097±0.001	0.181±0.002	0.188±0.001	0.175±0.002
4	*Bacillus licheniformis*	48hrs	0.232±0.002	0.370±0.003	0.354±0.003	0.422±0.003
		96hrs	0.198±0.003	0.280±0.001	0.187±0.002	0.376±0.001
5	*Pseudomonas aeruginosa*	48hrs	0.453±0.004	0.303±0.001	0.566±0.002	0.678±0.001
		96hrs	0.256±0.004	0.222±0.005	0.499±0.002	0.524±0.002
6	*Pseudomonas fluorescence*	48hrs	0.336±0.005	0.374±0.006	0.434±0.004	0.454±0.003
		96hrs	0.178±0.003	0.268±0.003	0.356±0.001	0.397±0.001
7	*Pseudomonas putida*	48hrs	0.297±0.002	0.276±0.003	0.387±0.004	0.377±0.005
		96hrs	0.196±0.001	0.205±0.003	0.310±0.005	0.305±0.002
8	*Staphylococcus aureus*	48hrs	0.304±0.003	0.279±0.005	0.398±0.006	0.649±0.003
		96hrs	0.273±0.002	0.223±0.002	0.330±0.001	0.565±0.005
9	*Enterobacteraerogenes*	48hrs	0.385±0.003	0.287±0.001	0.428±0.001	0.315±0.003
		96hrs	0.313±0.005	0.129±0.003	0.395±0.004	0.286±0.004

10	*Escherichia coli*	48hrs	0.380±0.006	0.388±0.003	0.310±0.003	0.485±0.004
		96hrs	0.292±0.002	0.315±0.003	0.274±0.002	0.386±0.006
11	*Acinetobacterbaumanii*	48hrs	0.295±0.001	0.321±0.001	0.216±0.002	0.406±0.001
		96hrs	0.228±0.004	0.286±0.005	0.179±0.004	0.375±0.005
12	*Enterobacter cloacae*	48hrs	0.395±0.003	0.351±0.005	0.477±0.002	0.468±0.003
		96hrs	0.329±0.004	0.294±0.003	0.454±0.005	0.427±0.006

Molecular Analysis

Gel Image of Exiguobacterium profundum

Plate 1 shows the PCR product of *Exiguobacterium profundum* that was produced after running the Gel electrophoresis.

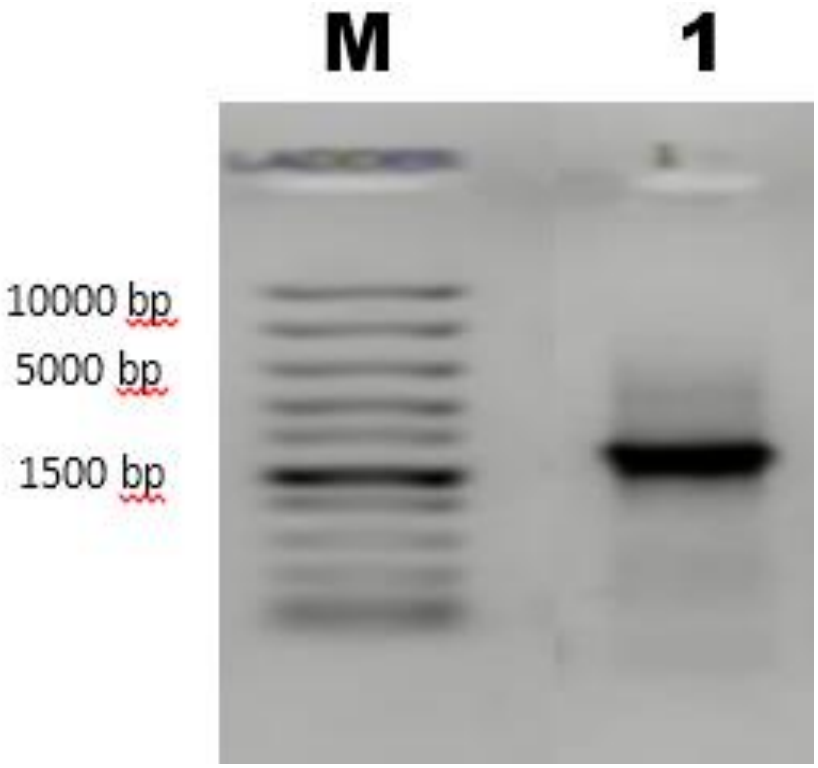

Plate 1: Gel image of *Exiguobacterium profundum*

Phylogenetic Analysis of 16S rRNA of Bacteria

The evolutionary relationships between different identified species and other species are depicted in Figure 1 of the phylogenetic analysis. The relationships are based on similarities and differences in the evolutionary genetic characteristics of the specie. And their related species from the GenBank of NCBI database. Their neighbor joining technique was used to create their phylogenetic tree using the nearby members found in NCBI GenBank.

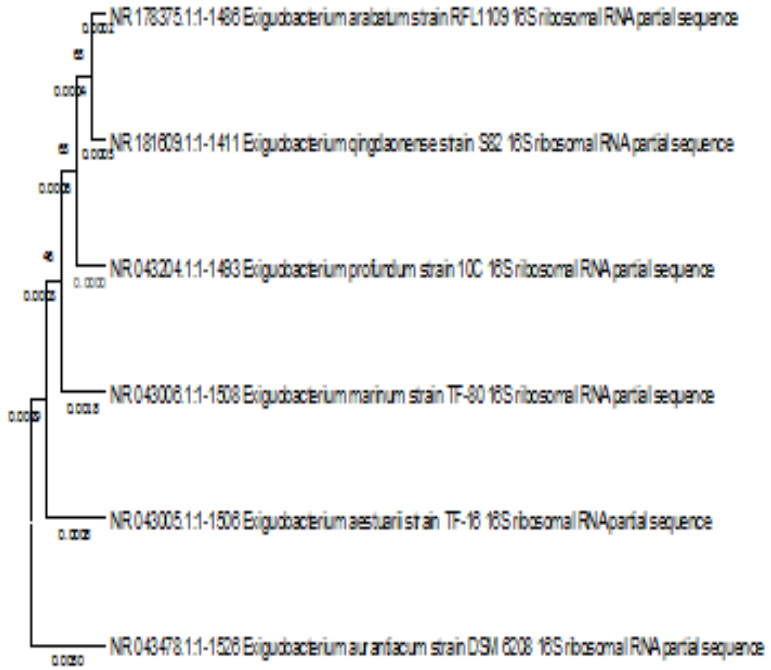

Figure 1: Phylogenetic Tree of *Exiguobacteriumprofundum*based on 16S rRNA Sequence using Neighbor Joining Method

Hit Similarity of the Most Potent Isolate(s) from GenBank of NCBI

Table 6 presents hit similarity of the most potent isolate(s) from GenBank of NCBI and the bacterium *Exiguobacterium profundum* was found to be highly similar with *Exiguobacterium profundum* strain 10C with 99.93% percentage identity as shown in Table 6.

Table 6: Hit Similarity of Isolates from GenBank of NCBI

Isolate	Best Hit Match	Percentage Identity	Accession No.
Exiguobacteriumprofundum	*Exiguobacteriumprofundum*strain 10C	99.93	NR043204.1

Discussion

The physicochemical parameters of the tannery effluents in Table 1 indicate severe pollution, as confirmed by their brown coloration and foul odour across all sampling sites. The acidic pH values (5.25–6.87) which is in line with the study of Adejube *et al*. (2018) suggest that effluents discharged into the environment may alter soil and water chemistry, reducing microbial diversity and affecting nutrient mobility. Similar studies on tannery effluents have reported acidic pH as a typical signature of chromium-laden industrial discharge. The high temperatures of the tannery effluents (34–37°C) further indicate ongoing biological and chemical reactions within the effluent, which may accelerate heavy-metal solubility. Electrical conductivity values exceeding 650 µS/cm reflect high ionic strength of the tannery effluent, which is consistent with the presence of salts and chromium compounds commonly used in leather processing. This agrees with the research of (Yusuf, 2004). BOD levels were also elevated, particularly in UKA and UKB, implying substantial organic load capable of depleting oxygen when discharged into natural ecosystems. This supports the notion of (Ake *et al*. 2022). The extremely high TDS and TSS values indicate poor effluent treatment and the possibility of long-term ecological damage. Collectively, these results show an effluent environment capable of supporting resilient or heavy-metal-tolerant microorganisms. This result is in agreement with Sugasini and Rajagopal (2015) who worked on characterization of physicochemical parameters and heavy metal analysis of tannery effluent.

Heterotrophic bacterial counts presented in Table 2 ranged from 1.32×10^7 to 1.75×10^7cfu/g is in line with the finding of Adejube *et al*. (2018) revealing that despite the toxic nature of tannery waste, a diverse and dense microbial community is still present. This suggests microbial adaptation to chromium-rich conditions. The higher count in the tannery effluent at Unguwar Karaye Site A (1.75×10^7cfu/g) may indicate fresher or more nutrient-rich effluent discharge, while lower counts in some sites may reflect harsher toxicity or differences in organic matter input. As stated by (Ake *et al.,* 2023), the ability of bacteria to survive at such densities implies strong selective pressure for metal-tolerant species, which is beneficial for bioremediation applications.

The characterization of bacterial isolate in the tannery effluent in Table 3 revealed a wide range of Gram-positive and Gram-negative bacteria, including *Bacillus* species, *Pseudomonas* species, *Enterobacter* species, *Staphylococcus aureus*, *Exiguobacterium profundum, Escherichia coli*, and *Acinetobacter baumanii*. The dominance of *Bacillus* and *Pseudomonas* aligns with previous studies conducted by Ake *et al*. (2023) that show these genera as common inhabitants of contaminated industrial waste due to their ability to form spores, produce enzymes, and tolerate metals. The presence of *Enterobacter* and *Acinetobacter* species indicates fecal contamination and opportunistic environmental survival. The metabolic diversity shown in sugar utilization, enzyme production, and biochemical reactions suggests that the isolated organisms possess physiological traits that favour metal transformation, sequestration, or enzymatic reduction. This aligns with the study of Chandra *et al*. (2011).

The frequency of occurrence of the bacterial isolates in the tannery effluent was illustrated in Table 4. *Bacillus subtilis* was the most frequently isolated species (21.1%), demonstrating its superior adaptability to chromium-polluted environments. This is in line with Hauwa *et al*. (2023). *Exiguobacterium profundum, Bacillus pumilus, Pseudomonas aeruginosa,* and *Staphylococcus aureus* each showed moderate occurrences (10.5%). The remaining isolates appeared at lower frequencies (5.3%), suggesting either lower tolerance or reduced competitive advantage. As reported by Fardami *et al*. (2025), the diversity pattern indicates that chromium stress supports microbial populations capable of sporulation, biofilm formation, oxidative-stress resistance, or metal efflux mechanisms.

The biosorption assay in Table 5 revealed significant variations in chromium-removal abilities among the isolates. *Exiguobacterium profundum* demonstrated exceptional chromium reduction, with drastic decreases in absorbance from 48 to 96 hours across all tested concentrations. This confirms its strong metal-binding, enzymatic reduction, or intracellular sequestration capabilities. This is in line with the study of (Alam and Malik, 2008). *Bacillus subtilis* and *Bacillus pumilus* also showed considerable biosorption, although less effective than *Exiguobacterium profundum*. *Pseudomonas aeruginosa* exhibited moderate removal but retained relatively high absorbance values at higher concentrations, indicating less efficient reduction at increased chromium load. This agrees with the research carried out by Plestenjak *et al*. (2022) and (Ilias *et al.,* 2011). The observed time-dependent decrease in absorbance across isolates confirms active microbial biotransformation of Cr (VI) to less toxic Cr (III). These findings strongly agreed with Kabir *et al*. (2018), indicating that indigenous bacteria from tannery effluents can effectively remediate chromium contamination through natural biological mechanisms.

The gel image presented in Plate 1 showed clear amplification of the 16S rRNA fragment of *Exiguobacterium profundum*, validating the success of molecular identification. This confirms the reliability of earlier biochemical analyses and establishes the isolate as a distinct chromium-tolerant species suitable for advanced bioremediation research. This aligns with (Carvalho *et al.,* 2025). As noted by Balakrishnan *et al*. (2022), molecular confirmation enhances the scientific validity of using this isolate in future bioengineering or field level bioremediation applications.

Figure 1 depicted the result of the phylogenetic analysis. The phylogenetic tree demonstrates the evolutionary relationship of the isolate with other *Exiguobacterium* strains in the NCBI database. Its close clustering with related strains signifies high genetic similarity and evolutionary conservation of functional genes responsible for metal tolerance. This supports the research conducted by Sonkar *et al*. (2024), which hypothesized that *Exiguobacterium* species possess inherent mechanisms enabling them to thrive in harsh industrial waste environments. Table 6 presented the hit similarity from NCBI database. The 99.93% identity match between the isolate and *Exiguobacterium profundum* strain 10C provides strong genomic evidence that the identified bacterium belongs to the same species. High sequence similarity validates the accuracy of molecular sequencing and confirms that the strain is a genuine chromium-tolerant variant. This genomic evidence supports its suitability as a candidate for chromium bioremediation technologies. This research is supported by Alam and Malik, (2008), Kabir *et al*. (2018) and Adejube e*t al*. (2018), highlighting the potential of bacterial species isolated from tannery effluent to bioremediate heavy metal pollution, particularly chromium.

CONCLUSION

The study concluded that the tannery effluent was highly polluted and contains high concentration of Cr that poses environmental and health threat. A total of twelve bacterial species belonging to diverse genera, including *Bacillus subtilis* had the highest percentage frequency of occurrence (21.1%), which was found to be the most predominant species among the isolates that were isolated from the tannery water samples. Followed by *Exiguobacterium profundum* (10.5%), *Bacillus pumilus* (10.5%), *Pseudomonas aeruginosa* (10.5%), *Staphylococcus aureus* (10.5%), *while Bacillus licheniformis* (5.3%), *Pseudomonas fluorescence* (5.3%), *Pseudomonas putida* (5.3%), *Enterobacter cloacae* (5.3%), *Enterobacter aerogenes* (5.3%), *Escherichia coli* (5.3%) and *Acinetobacter baumanii* (5.3%) were the least prevalent.*Bacillus,* were isolated and identified. The

chromium biosorption assay demonstrated variable but promising reduction capacities among the isolates. *Exiguobacterium profundum* emerged as the most potent chromium-removing bacterium, exhibiting remarkable decreases in Cr(VI) absorbance over 96 hours across all concentrations. Other species, such as *Bacillus subtilis, Bacillus pumilus*, and *Pseudomonas aeruginosa*, also showed appreciable biosorption potential. Molecular identification using 16S rRNA sequencing provided definitive confirmation of the most efficient isolate, revealing 99.93% identity with *Exiguobacterium profundum*strain 10C. Overall, this study demonstrates that indigenous microorganisms present in tannery effluents possess remarkable chromium-tolerance and biosorption capabilities.

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