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# HEAVY METAL TOLERANT BACTERIA ISOLATED AND DETECTED FROM THE EFFLUENT OF HAZARIBAGH TANNERY INDUSTRY IN DHAKA CITY

*Md. Monirul Islam[1*], Sheikh Rashel Ahmed[2], Pronabananda Das[1], Md. Monirul Islam[1], Md. Liakat Hossain[3], M. H. Kabir[1], A. N. K. Mamun[1]*

***Address (es):*** S. Julius Oladeji,
[1] Plant Biotechnology and Genetic Engineering Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, G.P.O. Box No-3787, Dhaka-1000, Bangladesh.
[2] Department of Plant and Environmental Biotechnology, Faculty of Biotechnology and Genetic Engineering, Sylhet Agricultural University, Sylhet-3100.
[3] Institute of Tissue Banking and Biomaterial Research, Atomic Energy Research Establishment, G.P.O. Box No-3787, Dhaka-1000, Bangladesh.

*Corresponding author: monirgebcu@gmail.com

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## ABSTRACT

The tanning industry is held to be an activity with the high budding for environmental pollution all over the world. Many Bacterial strains isolated from natural resources have been found to possess unique properties which make them useful for environmental cleans ups. So it is very important to find out an alternative ecofriendly way for the treatment of contaminated effluent. The objective of this study was to isolate, explore and pick out natural occurring bacteria capable of reducing heavy metals from tannery effluent collected from the Hazaribagh tannery industry of Dhaka. The pH value of all the effluents samples were ranged from 7.12 to 7.91. Five bacterial strains were confirmed as *Bacillus bataviensis*, *Bacillus aryabhattai*, *Micrococcus antarcticus*, *Bacillus proteolyticus* and *Bacillus paranthracis* on the basis of their morphological, cultural, biochemical, and 16S rRNA gene sequencing. Among these five strains, *Bacillus bataviensis*D1 exhibited higher resistance to cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and arsenic (As) up to the amount of 550 µg/mL, 500 µg/mL, 500 µg/mL, 1050 µg/mL and 1100µg/mL respectively. *Bacillus aryabhattai* D2 and *Micrococcus antarcticus* D3 showed similar result to chromium (Cr) and Lead (Pb), but *Bacillus proteolyticus* B1 showed higher resistance to nickel (Ni) that is up to 250 µg/mL. From these results, it can be suggested that the identified heavy metal-adapted bacteria could be useful for the biosorption of heavy metal contaminated effluent.

**Keywords:** Bacterial strains, Tannery effluent, 16S rRNA sequencing, Heavy metals, *Bacillus sp*, *Micrococcus sp*.

## INTRODUCTION

In Bangladesh government has identified the leather industry as one of the promising sectors with considerable growth and investment potential which secured fifth position in getting export currency. There are many factors existing in Dhaka city such as unavailability of proper facilities to manage tannery wastes, having inadequate knowledge regarding green industrialization threaten the quality of environment. Most of the tannery industries are discharging their wastes in the rivers and also in the surrounding soils that results in water pollution. Our country belongs to more than 113 tanneries in with an annual processing capacity of 180 million square feet of hides and skins. Most of the tanneries do not have proper effluent plants and generate nearly 20000 $m^3$ tannery effluents and 232 tones solid waste per year (**Paul *et al.*, 2013)**. From old times, tanning activities were organized to meet the local demands of leather shoes, belts, bags, upholstery, gloves, drums and musical instruments. The increasing demand of leather and its product lead to the increasing number of commercial tanneries globally. Tannery effluent is highly polluted because it contains imbalance suspended solids particles, electrical conductivity (EC), nitrogen, sulfide, sulfate and copper (Cu), chromium (Cr), cadmium (Cd) and manganese (Mn), chemical oxygen demand (COD) and biological oxygen demand (BOD) (**Mondal *et al.*, 2005; Zahid *et al.*, 2006**). Although some heavy metals are essential for life's physiological processes, their lavish accumulation in living organisms is always inimical. Various microbial growth are greatly hampered resulting in the abnormality in biomass and biodiversity **(Ayangbenro and Babalola, 2017**; **Roane and Pepper, 2000**). Lower number of microbes reside in the habitats with higher level of metal contamination than the uncontaminated habitats (**Tscherko *et al.*, 2000**). Heavy metals which are commonly used for the production of color pigments of dyes are chromium (Cr), copper (Cu), cadmium (Cd) and lead (Pb) (**Halimoon and Yin, 2010**). The impact of Zinc, Cadmium, and Mercury on microbial metabolism is dependent on the growth form, while it is consortia from mining sites; the resistance thresholds are lower in pure culture than in consortium (**Sprocati *et al.*, 2006**). Chlorinated organic compounds and heavy metals are major contaminants found in the environment, are extremely lethal to microbes, plants animals and human(s), which can destroy the DNA structure by damaging cell membrane. This toxicity is created by the rearrangement of elemental metals from their native binding sites or ligand interactions (**Olaniran *et al.*, 2013**).
There are some microbiological parameters such as the weight, number, and activity of microorganisms can be a good indicator of soil contamination with heavy metals (**Brookes, 1995**). Heavy metals have unusual attributes that they do not decay with time; they can be crucial or useful to plants at a certain level but can be poisonous when transcending lower limit; they strongly interact with the soil matrix consequently; heavy input in soils being related to weathering of parent rocks and pedogenesis; and they frequently present as cations which metals in soils can become portable as a results of changing habitat conditions. This situation is regarded to as "Chemical timing bomb" (**Facchinelli *et al.*, 2001)**. Tannery wastewaters are generally featured by high organic loading, high salinity, and certain pollutants such as Chromium (**Tunay *et al.*, 1994; Song *et al.*, 2000**). In tanning industries, many chemicals (salt, soda ash, lime, ammonium sulfate, ammonium chloride, fat liquors, sodium bicarbonate, sodium carbonate, chrome sulfate, sodium sulfate, magnesium oxide, vegetables oils and dyes) are commonly used to transform hide and skin of animal into commercial product. Alongside these, chemicals such as mercuric chloride, zinc chloride, and formaldehyde are used as disinfectants; sodium chloride in curing and as bleaching powder and sodium fluoride to prevent putrefaction; sodium sulfate, ammonium chloride, borax and hydrochloric acid in deliming agent; lime in liming; sodium for decreasing and various acidic or basic dyes are also used to process leather as finished product **(Saritha and Chockalingam, 2018**). A rigorous and substantial ruin of the environment is another point of concern from such a focal industry to sustain a billion dollar business due to the lack of a better and efficient waste water treatment. An excess of such chemicals in the water and soils is detrimental to the health of the people guzzle into the area (**Sundar *et al.*, 2010**). Several microorganisms have developed the process of removing poisons from a substance and respiration mechanism using heavy metals and thus become resistant to it (**Ezaka and Anyanwu, 2011**). Because of its metal accumulation ability and its resistance properties, the isolation and detection of heavy metal tolerant bacteria are crucial for removing and detoxifying of toxic metals. Therefore, the study focuses on isolation, biochemical and molecular characterization of naturally residing tannery effluent's bacteria, their characterization of heavy metal tolerance, in order to use them for detoxification in an incorporated biosorption strategy.

## MATERIALS AND METHODS

The study was conducted in Soil and Environment Laboratory, Biological Research Division, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh.

### Sampling sites

Hazaribagh tannery located at Dhaka city was selected for the collection of Tannery effluent samples (Fig. 1). Pre-sterilized bottles, used to keep the

collected samples, were well sealed with plastic bags and transferred very quickly to the BCSIR laboratory in ice-box; collected samples were preserved at 4ºC before analysis and during experiments.

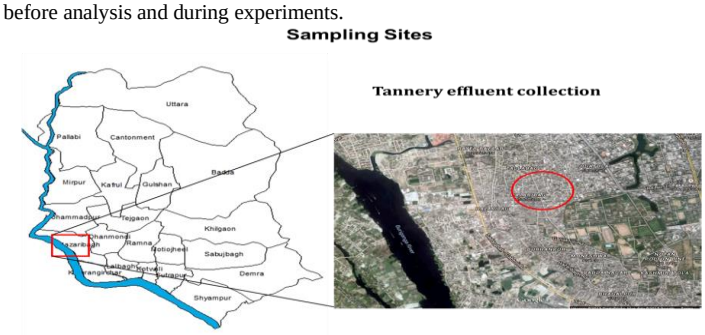


**Figure 1** Image of the location of Hazaribagh Tannery industrial areas

**Measurement of pH of the Samples**

The pH of the effluent was measured immediately in the laboratory with pH meter (Sension Refillabe pH electrode, HACH) after transportation of the samples to the laboratory.

**Isolation of Bacteria**

Serial dilution plate techniques were carried out and subsequent serial dilutions were made up five times for the isolation of bacteria. Nutrient agar plates were seeded with each diluted samples in duplicate and incubated at 37ºC for at least 24h in an incubator (**Zahoor and Rahman, 2009; Sujatha *et al.*, 2012; Ezaka *et al.*, 2011**). A total of twenty-five isolates were isolated from colonies differing in morphological characteristics; among them five isolates were selected and used for further analysis. The purification of selected isolates was done by streak plate method through repeated plating (**Greenberg *et al.*, 1980**) and the identification of that isolates were performed based on gram staining methods, colony characteristics and by various biochemical tests as described by Bergey's (1974) Manual of Determinative Bacteriology.

**Maintenance of Isolates**

The fumigated isolates were then transplanted on nutrient agar slant. The slants were maintained as a stock culture in a refrigerator at 4ºC for further study. Periodical transfer of isolates on agar slants was done for maintaining the viability of the organisms after each week (**Cappuccino and Sherman, 2005**).

**Physiological and Biochemical studies of Isolates**

Selected bacterial strains were grown in nutrient broth culture medium containing 2.5% peptone, 1% yeast extract and 0.5% beef extract. Cultures (50 ml in 250 ml conical flasks) were inoculated with 5% (v/v) inoculums and incubated at 37ºC for 24h with uninterrupted vigorous orbital shaking at 150-180 rpm. After gram staining, we used a microscope to examine the color and shape of vegetative cells of selected strains. Whether the arrangement of cells exists in singles or in chains or clusters was carefully recorded. Isolates were physiologically and biochemically analyzed to identify unknown isolates for the activity of Oxidase, Catalase, Acid gas production from D-glucose, Hydrolysis of starch, Liquefaction of gelatin, Indole formation, MR test, VP test, Deamination of Phenylalanine, and Citrate utilization (Aneja, 2003). These tests were performed using Bergey's Manual of Determinative Bacteriology along with other manuals such as Manuals of Microbiological Methods (**SAB, 1957**), Microbiological Methods (**Collins and Lyne, 2004**).

**Bacterial Genomic DNA isolation**

Bacterial genomic DNA was primarily isolated as per the standard protocol (**Sohail, 1998**). It was then visualised on gel electrophoresis at 0.8% agarose gel with ethidium bromide (0.5 µg/mL) as per the standard protocols and compared with marker DNA to assess genomic DNA.

**Automated sequencing and bioinformatics analysis**

For molecular characterization, sequence analyses of 16S rRNA gene were performed by PCR, using the bacterial universal primers (Forward primer 5′-CCAGACTCCTACGGGAGGCAGC-3′ and reverse primer 5′-CTTGTGCGGGCCCCCGTCAATTC-3′) for the amplification of the 16S rRNA gene fragment. PCR amplified DNA of the 5 isolates was purified using PCR Purification Kit by silica- gel membrane absorption method and sent for automated sequencing (Applied Bio-system 3130) to the Centre for Advanced Research in Sciences at University of Dhaka. 16S rRNA gene sequences were conveyed into "Basic Local Alignment Search Tool (BLAST)" available from the website of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to distinguish matches with existing identified reference sequences. The result of BLAST searches was sorted based on highest matches with other genus or species names in Genbank records. The phylogenetic trees were constructed based on the 16S rRNA sequences of each bacterium with reference sequence in Genbank using CLC drug discovery workbench software version 1.0.2 to define the identities and conserved region (**CLC Inc A, Denmark**).

**Screening of multiple heavy metal tolerant bacteria**

For the selective screening of heavy metal tolerant bacteria, various concentrations (10 µg/mL -100 µg/mL) of each heavy metals i.e. Cadmium Nitrate (Cd(NO3)2), Chromium (III) Nitrate(Cr(NO3)3), Nickel Nitrate (Ni(NO3)2), Cupric Sulphate ($CuSO_4 \cdot 5H_2O$), Lead (II) Nitrate (Pb(NO3)2) and Sodium Meta Arsenate (NaAsO2) were incorporated on to LB (Luria Bertani) agar plates and screened by the standard spread plate method (**APHA, 1992**) observed at 37ºC/24h for any kind of development on the culture medium. After the primary screening of effluent samples containing heavy metal resistant isolates, a serial dilution was done as (**Greenberg *et al.*, 1980**) to isolate desired bacteria. Streak plate technique was followed during isolation. Controls plates also prepared with LB media without including any heavy metal to make a comparison. Colonies differing in morphological attribute were selected, picked, purified and then maintained on different plates for further studies.

**Assessment procedures of minimum inhibitory concentration (MIC)**

For the determination of MIC, selected heavy metal tolerant isolates were grown on heavy metal associated media against respective heavy metals (Cd, Cu, Cr, Ni and Pb) with varying concentrations ranged from 50 to 1200 µg/mL with gradual increase of the heavy metal concentration by 50 µg/mL each time on nutrient-agar plate. The starting concentration of heavy metals was 50 µg/mL and culture growing on last concentration was transferred to higher concentration each time while streaking on the agar plate. This method was based on the procedure described by Yamina et al (2012) with slight modification. The cultures were incubated for 24-72h and measured for optical density (OD) at 620nm in UV spectrophotometer. A culture, having an OD greater than 0.1 at 620nm, was considered resistant. MIC was determined according to standard protocol of European Food Safety Authority (**EFSA**), Parma, Italy, 2012 when the isolates failed to grow on petri dish.

**RESULTS**

**pH measurement of effluent samples**

Physical and chemical properties of effluents have partial effect on the resistance of the microorganisms (**Kermanshahi *et al.*, 2007**).

**Table 1** pH value of the effluents samples

| Sample No. | Location | pH |
|---|---|---|
| D1 | Tannery Area, Hazaribagh | 7.46 |
| D2 | Tannery Area, Hazaribagh | 7.30 |
| D3 | Tannery Area, Hazaribagh | 7.12 |
| DY | Tannery Area, Hazaribagh | 7.91 |
| B1 | Tannery Area, Hazaribagh | 7.86 |

Since the resistance of the microorganisms would be affected by the variation of pH and the pH of the effluents samples were near about neutral, there would be

little effect on bacterial resistance. As given in table 1, the pH values of five respective samples varied from 7.12 to 7.91.

**Isolation of heavy metal resistant bacteria**

Being an industrial city, Dhaka is facing pollution problems. For example, Tanning and other industries discharge various types of heavy metals those are very toxic and accumulate in food chain, inhibits metabolic reactions causing the collapsion of marine biodiversity in nature. Prefatory screening of the collected samples for heavy metal tolerance ability showed that all samples were positively grown utilizing heavy metals ($Cu^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Cr^{6+}$, $Pb^{2+}$, and $As^{2+}$) in culture media. Preliminary screening of the characterization of bacteria were found belong to five bacterial species as *Bacillus bataviensis*, *Bacillus aryabhattai*, *Micrococcus antarcticus*, *Bacillus proteolyticus* and *Bacillus paranthracis* based on morphological characteristics (gram reaction, colony color, shape and arrangement of cells, motility) and biochemical characteristics (Oxidase test, Catalase Test, Oxygen Requirement, Gelatin Liquefaction, Starch Hydrolysis, Indole Formation VP Test, MR Test, Deamination of Phenylalanine, Utilization of Citrate, Acid Production from D-Glucose) which are mentioned in Table 2.

**Table 2** Morphological and Biochemical Characteristic of bacterial isolates

| **Morphological Characteristics** | **Bacterial isolates** | | | | |
|---|---|---|---|---|---|
| | **D1** | **D2** | **D3** | **B1** | **DY** |
| Gram reaction | + | + | + | + | + |
| Colony color | Cream | Initially brown, later peach | Yellowish | Pink | Off White |
| Shape and arrangement of cells | Rod, rounded end, occur in chain | Rod shaped rounded | Spherical, occur in pairs and packets | Rod shaped | Rod, occur in chain |
| Motility | + | + | - | + | - |
| **Biochemical test result** | | | | | |
| Oxidase test | - | + | + | + | - |
| Catalase Test | + | + | + | + | - |
| Oxygen Requirement | Facultative anaerobes | Facultative anaerobes | Strictly aerobes | Facultative anaerobes | Facultative anaerobes |
| Gelatin Liquefaction | + | + | + | + | + |
| Starch Hydrolysis | - | + | + | + | + |
| Indole Formation | - | - | + | + | + |
| VP Test | - | + | + | - | + |
| MR Test | + | - | + | - | - |
| Deamination of Phenylalanine | - | - | - | + | - |
| Utilization of Citrate | - | - | + | + | + |
| Acid Production from D-Glucose | + | + | - | + | + |
| Name of the Isolates | *Bacillus bataviensis* | *Bacillus aryabhattai* | *Micrococcus antarcticus* | *Bacillus proteolyticus* | *Bacillus paranthracis* |

**Assessment of MIC against each heavy metal**

The effluents sampled from tannery area had higher concentration of $Cu^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Cr^{6+}$, $Pb^{2+}$, and $As^{2+}$. It is mentioned that the isolates those showed high resistance to Cr, Cd, Cu, Pb and As were isolated from contaminated sites. The multi-metal resistance capacity approached two bacterial isolates *Bacillus bataviensis* and *Micrococcus antarcticus* are highly resistant to Cd, Cu, Pb, and As compared to *Bacillus paranthrac* which is reported in Table 3. Minimum inhibitory concentration (MIC) is the lowest concentration of the metal at which the isolate is completely suppressed (**Roane and Kellogg, 1993**). In this study, the metal resistant tests showed that some of the selected isolates had MIC above 600 µg/mL against cadmium (Cd); above 550 µg/mL against chromium (Cr); above 300 µg/mL against Nickel (Ni); above 450 µg/mL against copper (Cu); above 1000 µg/mL against lead (Pb), and above 1100 µg/mL against arsenic (As).

The MIC of identified strains for $Cr^{6+}$ ranged from 400 to 600 µg/mL, for $Cd^{2+}$ ranged from 300 to 650 µg/mL, for $Ni^{2+}$ ranged from 250 to 350 µg/mL, for $Cu^{2+}$ ranged from 400 to 500 µg/mL, for $Pb^{2+}$ ranged from 600 to 1050 µg/mL, for $As^{5+}$ ranged from 900 to 1150 µg/mL in nutrient broth**.**

In this study order of bacteria tolerance to Cadmium and Arsenic was found to be *Bacillus bataviensis*>*Micrococcus antarcticus*>*Bacillus proteolyticus*>*Bacillus aryabhattai*>*Bacillus paranthracis* and *Bacillus aryabatti*> *Bacillus bataviensis*> *Bacillusparanthracis*> *Bacillus proteolyticus*>*Micrococcus antarcticus.* Upon above experiment, the resistance level *Bacillus bataviensis*> *Bacillus proteolyticus*>*Bacillus aryabhattai*= *Bacillus paranthracis*> *Micrococcus antarcticus* showed for Pb and *Bacillus bataviensis*>*Bacillus aryabatti*= *Micrococcus antarcticus*> *Bacillus paranthracis*> *Bacillus proteolyticus* showed for Cr. Our study found multiple heavy metal tolerance capacity for *Micrococcus antarcticus* having MIC 950 µg/mL, 500 µg/mL, 400 µg/mL and 900 µg/mL against As, Cr, Cu and Pb respectively (Table 3). Among five isolates bacteria, *Bacillus aryabatti* and *Micrococcus antarcticus* were identified here as the lowest resistance to Nickel.

**Table 3** Heavy metal resistance capacity and MIC of bacterial isolates

| Resistance capacity | Strain No. | | | | |
|---|---|---|---|---|---|
| | **D1** | **D2** | **D3** | **B1** | **DY** |
| Cd | ++ | + | ++ | + | + |
| Cr | ++ | ++ | ++ | + | + |
| Ni | + | + | + | + | + |
| Cu | ++ | + | + | ++ | + |
| Pb | +++ | ++ | ++ | +++ | ++ |
| As | +++ | +++ | ++ | +++ | ++ |
| **MIC (**µg/mL**)** | | | | | |
| Cd | 650 | 400 | 500 | 450 | 300 |
| Cr | 600 | 500 | 500 | 400 | 450 |
| Ni | 300 | 250 | 250 | 300 | 350 |
| Cu | 500 | 450 | 400 | 500 | 450 |
| Pb | 1050 | 950 | 900 | 1000 | 950 |
| As | 1100 | 1150 | 950 | 1000 | 900 |

+++ Indicates-High resistant, ++ indicates- Moderate and + indicates- Low

**Analysis of 16S rRNA gene sequencing results**

Comparative analysis of the sequences with already available database showed that the strains were closed to the members of genus *Bacillus* and *Micrococcus* (Fig. 2). To detect the possible similarity of organisms via the alignment of homologous sequences, NCBI BLAST (**http://blast.ncbi.nlm.nih.gov/**) program was subjected to analyze the sequence resulted from the automated sequencing PCR amplified DNA (**Marchler-bauer *et al*., 2002; Pruitt *et al*., 2005**)

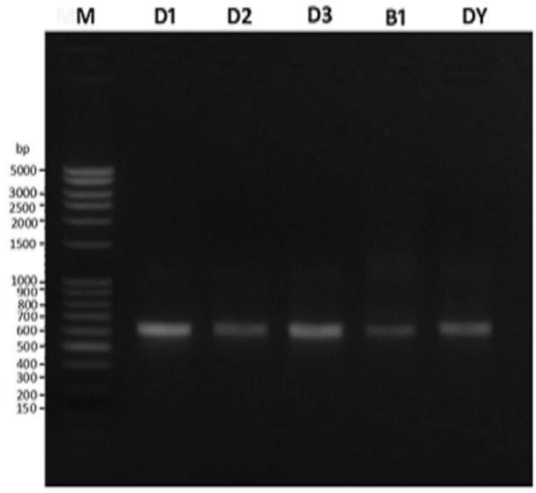


Figure 2 Gel image of 16S rDNA Amplicon, Line 1: 100 bp of DNA Marker [Lane 2, 3, 4, 5, 6: 16S rDNA Amplicon band of D1, D2, D3, B1, and DY isolates].

The highest sequence similarities of effluent bacteria are as follows: D1, *Bacillus bataviensis* strain NBRC102449 (Query coverage 94% and 99% similarity, accession number NR_114093.1); D2, *Bacillus aryabhattai* strain B8W22 (Query coverage 96% and 99% similarity, accession number NR_118442.1); D3, *Micrococcus antarcticus* strain T2 (Query coverage 93% and 99% similarity, accession number NR_025285.1); B1, *Bacillus proteolyticus* strain MCCC 1A00365 (Query coverage 96% and 99% similarity, accession number NR_157735.1); DY, *Bacillus paranthracis*strain MCCC 1A00395 (Query coverage 93% and 98% similarity, accession number NR_157728.1). All the closely related homologs of identified bacteria were used for the construction of the phylogenic dendrogram to know their evolutionary origin. The dendrogram showing the relation among *Bacillus bataviensis, Bacillus aryabhattai, Micrococcus antarcticus, Bacillus proteolyticus, Bacillus paranthracis,* and the close homolog's of them (fig. 3.(a-e))

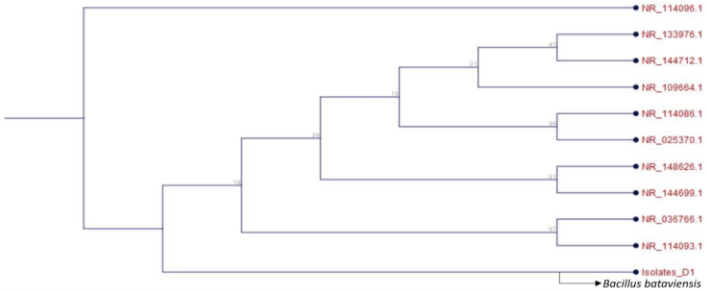


**Figure 3 (a)** Phylogenetic tree representing close homolog's of D1, *Bacillus bataviensis* strain NBRC 102449

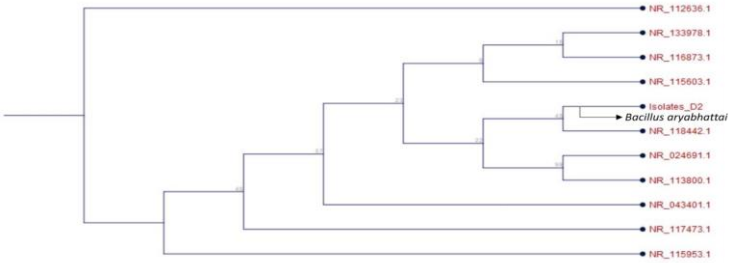


**Figure 3 (b)** Phylogenetic tree representing close homolog's of D2, *Bacillus aryabhattai strain B8W22*

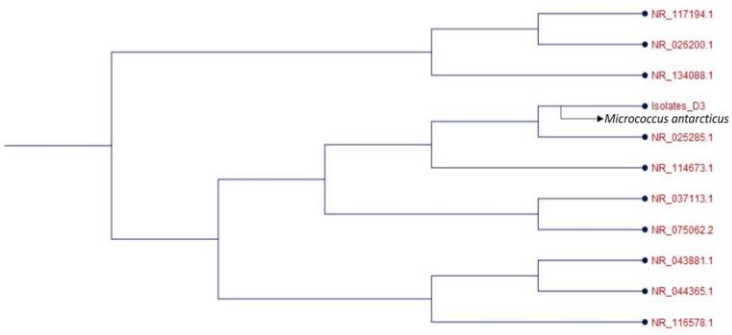


**Figure 3 (c)** Phylogenetic tree representing close homolog's of D3, *Micrococcus antarcticus* strain T2

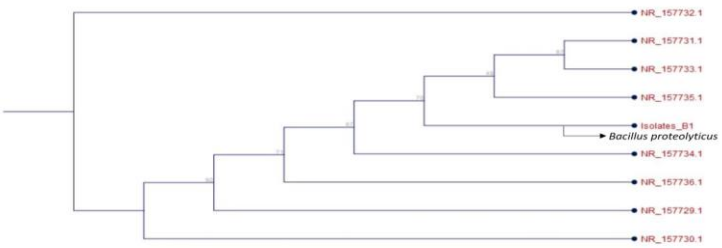


**Figure 3 (d)** Phylogenetic tree representing close homolog's of B1, *Bacillus proteolyticus* strain MCCC 1A00365

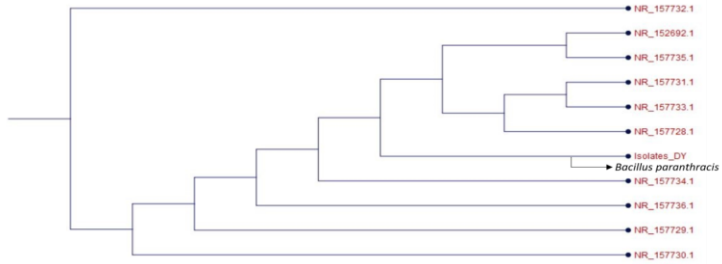


**Figure 3 (e)** Phylogenetic tree representing close homolog's of DY, *Bacillus paranthracis* strain MCCC 1A00395

## DISCUSSION

In the present study, bacterial strains were isolated from the most common heavy metal contaminated sites of tannery areas in Hazaribagh, specially focused on the identification of Cupper (Cu), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Arsenic (As) and Lead (Pb) tolerant bacteria in search of their metal resistance and minimum inhibitory concentration (MIC). pH value was observed in sample D1,D2, D3, B1, DY collected from tannery area, Hazaribagh and the pH value were found 7.46, 7.30, 7.12, 7.91, and 7.86 respectively. Therefore, high pH is one of the key characterization factors of the tannery waste (**Kumar and Mani, 2007**). The pH is one of the parameters of effluents by which the microbial activity is greatly affected. pH influences all the enzymatic reaction of microbial metabolism and so resistance of microbes may be influenced. The observed pH values were almost nearer to neutrality. So, microbial activities are not much more affected (**Kermanshahi, *et al*., 2007**).

The gram staining test showed that all isolates were gram-positive (Table 2). *Micrococcus sp*. is strictly aerobes and oxidase-positive, which can be used to differentiate them from other gram-positive bacteria like most *Staphylococcus sp*. (**Thelwell *et al.*, 1998**), which are normally oxidase negative. In our experiment, D3 isolates was identified as oxidase positive as well as it may be *Micrococcus antarcticus* (**Liu *et al*., 2018**). In growth medium, it generated yellow to brown colonies, on the contrary, *Micrococcus roseus* produced red colony (Table 2). *Bacillus proteolyticus* being a protease producing bacteria has the possible activity for use in aquaculture as a bioremediation (**Bhaskar and Sudeepa, 2007**). *Bacillus anthracis* is a member of the bacillus cereus group species that are non-punctilious facultative anaerobes and gram-positive spore forming bacteria (**Koehler, 2009**).

**Sanjay *et al*., (2018)** isolated two bacterial species *Klebsiella pneumoniea* strain MS 1.5 and *Mangrovibacter vixingensis* Strain MS 2.4 that showed high reducing ability for Cr (VI) upto 80 mg/l and 100 mg/l respectively. **Rajbanshi (2008)** also reported that the results of minimum inhibitory concentration of different bacteria 150 to 500 µg/mL for chromium and 200 to 300 µg/mL for copper. **Brocklehurst and Morby (2000)** reported that in response to toxic concentrations of heavy metal ions, Escherichia coli strain exhibited varying degrees of tolerance (3 to 14-fold) both to the adaptive metal and its congeners. **Singh *et al*., (2010)** isolated a bacterium *Bacillus cereus*, which exhibited a high degree of tolerance to heavy metal like lead, arsenic, and cesium.

In our experiments, the resistant level As>Pb>Cd>Cr>Cu>Ni showed for the maximum isolates. In this study order of MICs for the isolates D1, D2 and D3 was found to beAs>Pb>Cd>Cr>Cu>Ni and; As>Pb>Cr>Cu >Cd>Ni, and As>Pb>Cd=Cr>Cu>Ni. The order of other isolates B1 and DY was found to be As>Pb>Cu>Cd >Cr>Ni and Pb>As>Cr=Cu>Ni. It was also demonstrated that the resistant levels of heavy metal for sewage bacteria *Proteus vulgaris*, *Acinetobacter redioresistens* and *Pseudomonas aeruginosa* were shown to be As>Pb=Ni>Cd>Cr>Hg on the LB agar plates (**Raja *et al*., 2009**). Besides *Micrococcus antarcticus* shows sensitivity to Cr and Pb as well as their resistance capacity against Ni and Cu are also lower compared to other bacteria.

## CONCLUSION

Genotypic characteristic for bacterial strain identification is accurate, simple and effective than identification by the conventional phenotypic method. We performed various biochemical characterization and molecular techniques; and identified *Micrococcus sp.* and *Bacillus sp.* This identification of bacterial species can be used to its multiple metal tolerant capacity and MIC which can be further used for the biosorption of heavy metal pollutants in the environment. In future, these bacteria can be employed for remediation of heavy metal released into the environment, used for extraction of novel metal and potential use to safeguard the ecosystem.

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