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Characterization and identification of bacterial pathogens from treated tannery wastewater

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ABSTRACT

The aim of this study was to isolate, characterize and identify the bacterial pathogens from treated tannery wastewater and also to study their multi-drug and multi-metal resistance property. Three bacterial strains of pathogen species with more antagonistic activity were identified as *Pantoea* sp*., E. coli* sp., and *Lysinibacillus* spp., with accession nos. *KJ576899, KJ576900* and *KJ576904*, respectively for public domain. The antibiotic and heavy metal resistant property of isolated bacterial strains was investigated by disk diffusion method on Muller-Hinton agar and nutrient agar medium amended with increasing concentrations of various toxic metal ions. The isolated bacterial strains *Pantoea* sp*.* was sensitive to *amikacin, ampicillin, cefepime, chloramphenicol, levofloxacin, meropenem, nalidixic acid, piperacillin* and resistant to for carbenicillin*, aztreonam, cefotaxime, ciprofloxacin, cotrimoxazole, moxifloxacin, streptomycin, tetracycline.* The other two bacterial strains of pathogen *E. coli* sp., and *Lysinibacillus* spp. showed different level of resistance and, sensitivity, to many antibiotic tested. In addition, the bacterial pathogens also showed the Minimum Inhibitory Concentration (MIC) of Cd, Cu, Cr, Co, Ni, Mn, Pb, Zn and Fe. These multi-drug and multi-metal resistant organisms can be used as a potential agent for the bioremediation of metal contaminated sites.

Keywords: Antibiotic, metal, bacterial pathogen, *E. coli* sp., *Lysinibacillus* sp., *Pantoea* sp.

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INTRODUCTION

Tannery industries are the backbone of world's economy because these industries manufacture leather footwear, jackets, drums and musical instruments etc., which is used at large scale (Durai and Rajasimman, 2011; Lofrano et al., 2013). Unfortunately tannery industry generate vary toxic and complex wastewater with unpleasant odor. Tannery industries used various processes such as hide and skin storage and beam house operation, tanyard operations, post-tanning and finally finishing of leather. In which, different chemicals are used till final process (Ozgunay et al., 2007). Leather industries mainly use animal hides. During processing, huge volume of water and large quantities of chemicals such as ammonium sulfate, vegetables tannins, sodium chloride, lime, sodium sulfide, bactericides etc. are used.

During the tanning process, chromium salt is used to convert hides into leather and the wastewater generated contains huge amount of organic matter, phenolics, tannins and toxic heavy metals mainly chromium (Ma et al., 2015; Kongjao et al., 2008; Nithya and Sudha, 2016). This wastewater containing a variety of toxic pollutants when discharged into the environment causes serious

and water pollution along with serious health hazards to humans, animals as well as plants and also create favorable environment for pathogenic and non-pathogenic microbes to flourish and contaminate the environments, whereas toxic metals induce genotoxic and mutagenic changes in bacterial communities making them resistant against a wide spectrum of antibiotics and toxic metals (Malik and Jaiswal, 2000; Ramteke et al., 2010; Viti et al., 2003; Flores et al., 2012; Paul et al., 2012).

In literature, sewage contamination is reported as a major source of pathogenic bacteria in water resources, but various national and international authors also suggested that besides sewage contamination, the wastewaters discharged from various industries such as distillery, pulp and paper mills and tannery industries etc. also act as a good source of nutrients (organic and inorganic) and support the growth of pathogenic microbes in receiving water bodies (Lofrano et al., 2013; Wang et al., 2014; Ramteke et al., 2010; El-Lathy et al., 2009; Singh et al., 2016). However, the detailed information about the pathogenic microbes remained in tannery wastewater even after the secondary treatment process is still not available so far. Hence, this study was aimed to characterize and identify the bacterial pathogens able to survive in tannery wastewater and also to study their multi-drug and multi-metal resistant characteristics.

MATERIALS AND METHODS

Collection of treated tannery wastewater

The treated tannery wastewater samples were collected in sterilized carboy container (capacity 10 L) from the Common Effluent Treatment Plant (CETP) of tannery industries located at Unnao, (26.48° N latitude, 80.43° E longitude), (U.P), India. The collected wastewater samples were brought to laboratory and stored at 4°C and used for the analysis of physico-chemical parameters as well as for the isolation of pathogenic bacteria.

Analysis of physico-chemical parameters

The collected tannery wastewater samples were analyzed for physico-chemical parameters according to standard methods for the examination of water and wastewaters in triplicate (APHA, 2012).

The collected wastewater samples were analyzed for the following parameters: pH, COD, BOD, total solids (TS), total suspended solid (TSS), total nitrogen (TN), sulfate (SO_4^{-2}) and phosphates (PO_4^{-3}) as well as the concentration of various toxic heavy metals (APHA 2012). The pH of collected tannery wastewater samples was measured by Orion ion meter (Model-960), BOD by 5 days method, COD by open reflux method, total solids (TS) by drying method, and total nitrogen (TN) by TOC-V_{csn} analyzer (Shimadzu, JAPAN). Whereas, phosphate and sulfate was measured by vanadomolybdo-phosphoric acid colourimetric and $BaCl_2$ precipitation methods, respectively. The concentration of different heavy metals (Cu, Cr, Ni, Zn, Fe, Mn and Pb) was measured using inductively coupled plasma spectrophotometer (Thermo Electron; Model IRIS Intrepid II XDL, USA) (APHA, 2012).

Isolation of bacterial pathogens

For the isolation of bacterial pathogens, the collected tannery wastewater sample was serially diluted to desired concentration and plated on the nutrient agar (NA)and MacConkey agar plates followed by incubation at 35°C for 48 h. The bacterial colonies appearing on both media were picked up and investigated for purity as well as morphological characteristics by microscopic observation.

Characterization and identification of isolated bacterial pathogens

Biochemical characterization

Cowan and Steel's Manual was followed for the morphological and biochemical characterization of isolated bacterial pathogens (Barrow and Feltham, 1993).

Molecular characterization

Preparation of genomic DNA and PCR amplification of 16S rRNA gene: The genomic DNA was prepared from the overnight grown culture of bacterial pathogens following the alkaline lysis method (Coelho et al. 2003). About 2 µl DNA was used to amplify the 16S rRNA gene using the forward and reverse primers 27F (5'-AGA GTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), respectively (Chandra et al. 2011). The reaction mixture contained 2 µl of template DNA, 1X PCR buffer, 200 µM of each dNTP, 3.0 mM $MgCl_2$, 25 pmol of primer, and 2.5 units of Amplitaq DNA polymerase (Merk, Biosciences, India) in a final volume of 50 µl. The thermocycling reactions were carried out by using Veriti® 96-Well Thermal Cycler (Applied Biosystems, USA) as: 30 cycles of denaturation at 94°C for 1 min, followed by annealing at 45°C for 1 min and extension at 72°C for 2 min (Bharagava et al., 2009). The PCR products were gel purified using gel extraction kit (Merk, Biosciences, India), and sequenced by using 27F primer. The partial sequences obtained were subjected to BLAST analysis using the online option available at www.ncbi.nlm.nih.gov/BLAST to get the closest neighbor of the isolated bacteria.

Construction of phylogenetic tree and nucleotide sequence accession number: Neighbor-joining method (MEGA version 4.0 software) was used for the construction of phylogenetic tree (Tamura et al., 2007). For bacterium RMB1, five strains of *Pantoea* sp. and five strains of *Enterobacterium* sp. were used whereas for RMB2, six strains of *Escherichia coli* and three strains of *Shigella* sp. were used for generation of phylogenetic tree. Further, the phylogenetic tree of RMB6 strains was prepared by using the eight strains of *Lysinibacillus* sp., one *Proteus* sp. and one *Firmicutes* bacterium. The 16S rRNA gene sequences of the closest related species used in phylogenetic tree preparation were downloaded from the GenBank and the nucleotide sequences of the isolated bacterial pathogens were also deposited in the GenBank public database under the accession number *KJ576899, KJ576900* and *KJ576904* for bacterial strains RMB1, RMB2 and RMB6, respectively for public domain.

Antibiotic resistance property of bacterial isolate

The antibiotic susceptibility testing of isolated bacterial pathogens was done by the disk diffusion method on Muller-Hinton agar medium against the following antibiotics: *amikacin* (30 mcg),

ampicillin (10 mcg), *carbenicillin* (100 mcg), *aztreonam* (30 mcg), *cefepime* (30 mcg), *cefotaxime* (30 mcg), *chloramphenicol* (30 mcg), *ciprofloxacin* (5 mcg), *cotrimoxazole* (25 mcg), *levofloxacin* (5 mcg), *meropenem* (10 mcg), *moxifloxacin* (5 mcg), *nalidixic acid* (30 mcg), *piperacillin* (100 mcg), *streptomycin* (10 mcg), and *tetracycline* (30 mcg) (HiMedia, Mumbai). The plates were swabbed with a faintly opalescent culture, and the antibiotic disks were applied followed by the incubation at 32°C for 24 h (Jain et al., 2009). The inhibition zone was measured after 24 h of incubation period and the isolated bacterial pathogens were classified as resistant, intermediate, or sensitive based on the zone size as per the standard antibiotic disc sensitivity testing method (DIFCO, 1984).

Determination of minimum inhibition concentration (MIC) of various toxic metals for isolated bacterial pathogens

The minimum inhibition concentration (MIC) of Cd, Cu, Cr, Co, Ni, Mn, Pb, Zn, and Fe for the isolatedbacterial pathogenswas determined in nutrient broth amended with the increasing concentrations (0 to 1000 µg ml^{-1}) of each of the above mentioned metals. The stock solutions of salts of $CdCl_2$, $CuSO_4$, $K_2Cr_2O_7$, $CoCl_2$, $NiCl_2$, $MnSO_4$, $PbNO_3$, $ZnSO_4$, and $FeCl_3$, for Cd^{2+}, Cu^{2+},Cr^{6+}, Co^{2+}, Ni^{2+}, Mn^{+2}, Pb^{3+}, Zn^{2+} and Fe^{3}ions, respectively were prepared in autoclaved Millipore water. The experiment was performed in 50 ml tubes containing 20 ml of autoclaved nutrient broth supplemented with the increasing concentrations of the metals and 100 µl of bacterial culture followed by incubation for 48 h at 35°C and 125 rpm in shaking incubator (Jain et al., 2009).

The bacterial growth was monitored by measuring the optical density at 600 nm. The tubes containing 20 ml of autoclaved nutrient broth supplemented with the increasing concentrations (0 to 1000 µg ml^{-1}) of above metal ions without bacterial culture were taken as control. The MIC of different toxic metals for the isolated bacterial pathogen was designated as the minimum concentration of metal ions at which no growth of the test organism was observed.

RESULTS AND DISCUSSION

Physico-chemical characteristics of treated tannery wastewater samples

The physico-chemical analysis of collected tannery wastewater samples revealed that it had high BOD, and COD values, high TS, and high concentration of sulfate, phosphate, organic compounds and of different heavy metals (Table 1). Our results are well collaborated with the previous studies made by various other authors (Chandra et al., 2011; Chandra et al., 2009). However, the physico-chemical characteristics of industrial wastewaters varies considerably from industry to industry depending upon the production size, processes adopted, chemicals used in various processes and consumption of water (Bharagava et al., 2014; Chandra et al., 2011).

Table 1. Physico-chemical characteristics of treated tannery wastewater.

Sr. no.	Parameters	Values
1.	pH	7.86 ± 0.35
2.	Temp.	32.66 ± 2.08
3.	EC	10.93 ± 0.20
4.	BOD	27266.67 ± 208.16
5.	COD	56000 ± 360.55
6.	TS	16205 ± 368.88
7.	TSS	3281.66 ± 91.69
8.	TDS	14103.33 ± 83.86
9.	Sulfate	18833.33 ± 305.50
10.	Phosphate	15300 ± 173.20
11.	Nitrate	26 ± 1
12.	Chloride	2238±45.07
13.	Potassium	902±6.92
14.	Phenol	693.33±12.58
15.	Heavy metals	
	Cu	1.48 ± 0.026458
	Cd	0.16 ± 0.015
	Zn	10.96 ± 0.076
	Ni	0.31 ± 0.020
	Fe	49.88 ± 0.27
	Mn	41.71±0.79
	Pb	0.35±0.03

All the values are means of three replicate (n = 3) ± SD in mg L^{-1} except colour (Co–Pt) and pH.

Bacterial pathogens present in treated tannery wastewater samples

On the MacConkey agar plates, some bacterial strains were appeared as smooth, punctuate, convex, and glistening colonies.

Biochemical characteristics of bacterial isolates

The microscopic observation of isolated bacterial pathogens have revealed that out of the three bacterial isolates, bacterium RMB1 and RMB2 was gram-negative (-ve) rods while bacterium RMB6 was gram positive (+ve) rod shaped. However, all bacterial strains were found to be motile in nature. Further, on the MacConkey agar plates, RMB2 appeared as smooth, punctuate, convex, glistening and lactose-fermenting organism. Further, based on the biochemical reactions, bacterium RMB1 and RMB2 was found to be catalase positive, oxidase negative, lipase positive while bacterium RMB6 was catalase negative and oxidase positive as shown in Table 2.

16S rRNA gene sequence analysis and phylogenetic relationship

The PCR amplified 1500 bp long 16S rRNA gene

Table 2. Morphological and biochemical characteristics of the isolated bacterial strains from tannery wastewater.

S. No.	Morphology	RMB1	RMB2	RMB6
1.	Motility	+ve	+ve	+ve
2.	Gram Stain	-ve	-ve	+ve
3.	Cell shape	Rod	Rod	Rod
	Biochemical characteristics			
4.	Cellulose	-ve	+ve	-ve
5.	Indole Test	-ve	+ve	+ve
6.	Gelatinase test	+ve	-ve	+ve
7.	Voges-Proskauer test	+ve	+ve	+ve
8.	Methyl red test	-ve	+ve	-ve
9	Hydrogen sulphide test	-ve	-ve	+ve
10.	Lipase test	+ve	+ve	+ve
11.	Lysine utilization	-ve	-ve	+ve
12.	Urease test	-ve	-ve	-ve
13.	Casein	+ve	+ve	-ve
14.	Oxidase	-ve	-ve	+ve
15.	Catalase	+ve	+ve	-ve
16.	Citrate Utilization	+ve	+ve	-ve
17.	Nitrate Reduction	+ve	+ve	-ve
18.	Starch Hydrolysis	-ve	-ve	-ve

(Figure 1A) sequences obtained from the bacterial pathogensRMB1, RMB2 and RMB6 have shown the closest relatedness with that of *Pantoea* sp. XJ3 (GU140078) (Figure 1B(a), *Escherichia coli* strain JCM24003 (AB548576) (Figure 1B(b) and *Lysinibacillus sphaericus* strain NBRC 3526 (AB680103) (Figure 1B (c), respectively.

Further, the phylogenetic tree for bacterial strains RMB1, RMB2 and RMB6 was constructed by using five different *Pantoea* sp., five *Enterobacteria* spp., six *Escherichia coli* spp., three *Shigella* spp., eight *Lysinibacillus* spp. and one *Proteus mirabilis* spp. Some sequences of non-related species were also included in the tree as control to demonstrate the linkage distance between the selected pathogenic strains. Hence, based on the 16S rRNA sequence similarity (> 99%), the bacterial strains RMB1, RMB2 and RMB6 were identified as *Pantoea* sp*., E. coli* sp. and *Lysinibacillus* spp*.* with accession nos. *KJ576899, KJ576900* and *KJ576904*, respectively.

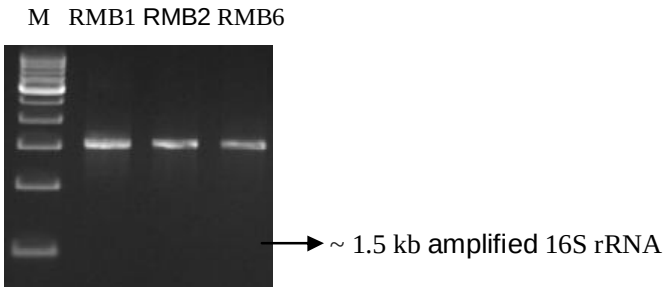

Figure 1A. PCR amplification of 16S rRNA gene and plasmid profiling of isolated bacteria (RMB1, RMB2 and RMB6); Lane 1: 500 bp Marker Ladder; Lane 2: PCR amplified 16S rRNA gene; Lane 3: Plasmid DNA; Lane 4: Supermix DNA Marker Ladder.

Antibiotic susceptibility of isolated bacterial strains

The results revealed that the isolated bacterial strain RMB1 was found to be sensitive to *amikacin*, *ampicillin*, *cefepime*, *chloramphenicol*, *levofloxacin*, *meropenem*, *nalidixicacid*, *piperacillin* and resistant to *carbenicillin*, *aztreonam*, *cefotaxime*, *ciprofloxacin*, *cotrimoxazole*, *moxifloxacin*, *streptomycin*, *tetracycline*. The other two strains RMB2 and RMB6 also showed to be resistance sensitivity, intermediate effect for many antibiotics as shown in Table 3. Our results were found somewhat similar to the previous findings made by other researcher also (Yadav et al., 2016; Bharagava et al., 2014; Abideen and Babuselvam, 2014).

Minimum inhibition concentration (MIC) of tested metal ions for isolated bacterial pathogen

The isolated bacterial strains also showed a wide range

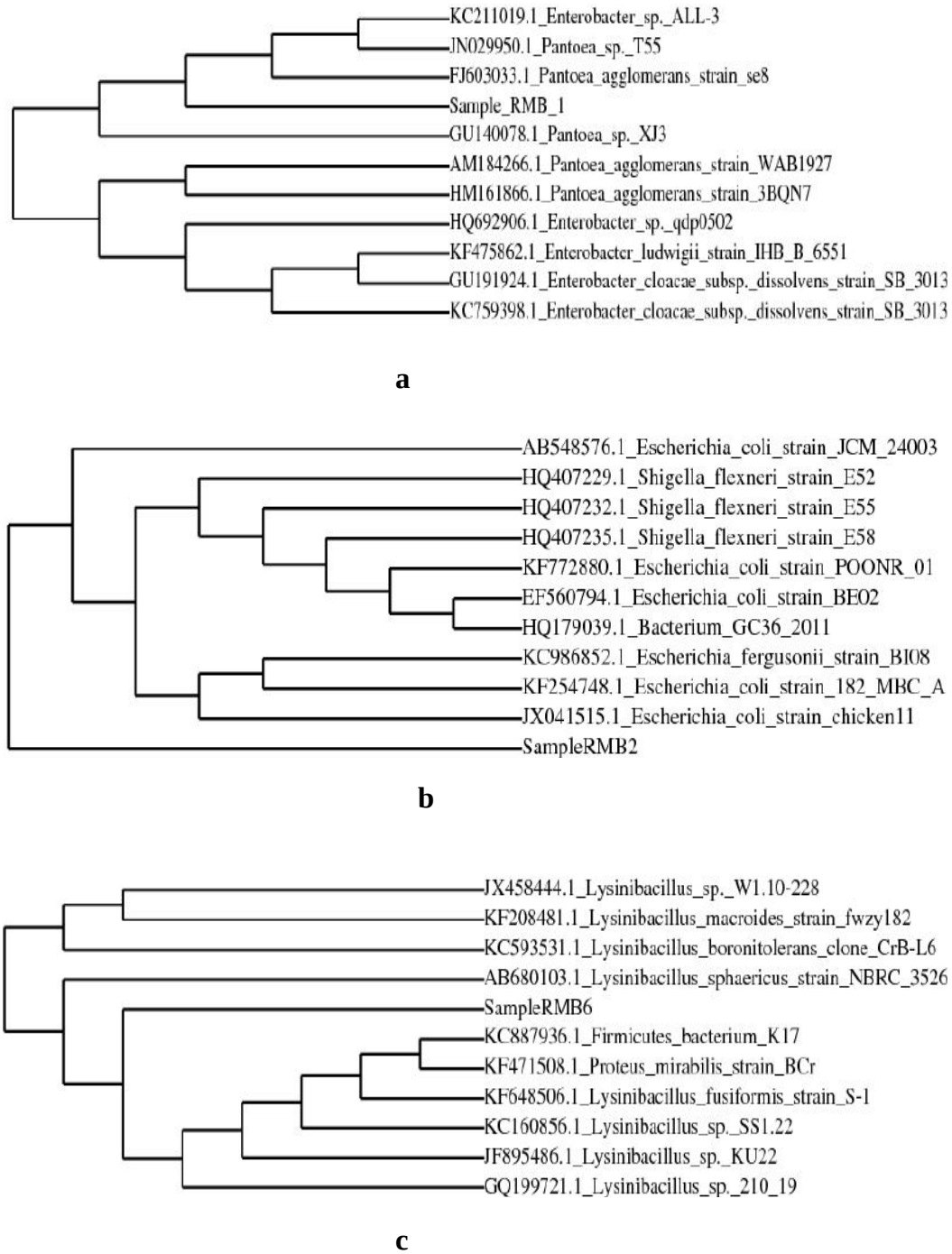

Figure 1B. Neighbor-joining tree showing the phylogenetic position of isolated pathogenic bacteria RMB1 (a), RMB2 (b), and RMB6 (c) with closest related species based on the 16S rRNA gene sequences. The GenBank accession number for each bacterium used in the analysis is shown in parenthesis before the species name.

of MIC values for the tested metals as presented in Table 4. However, the bacterial resistance to heavy metals is an important consideration when bacteria are to be introduced into the contaminated environments for enhancing the bioremediation of metal contaminated soils. Although some heavy metals are required in low concentrations for normal metabolic activities, at elevated levels, these metals act as carcinogenic, mutagenic or

Table 3. Antibiotic susceptibility pattern of isolated bacterial strains (RMB1, RMB2 and RMB6) from tannery wastewater.

Sr. no.	Antibiotics used for susceptibility test	Susceptibility pattern of isolated pathogenic bacteria		
		RMB1	RMB2	RMB6
1.	Amikacin	S	S	R
2.	Ampicillin	S	R	S
3.	Carbenicillin	R	S	R
4.	Aztreonam	R	R	S
5.	Cefepime	S	R	R
6.	Cefotaxime	R	S	R
7.	Chloramphenicol	S	S	S
8.	Ciprofloxacin	R	R	R
9.	Cotrimoxazole	R	I	R
10.	Levofloxacin	S	R	R
11.	Meropenem	S	S	R
12.	Moxifloxacin	R	R	S
13.	Nalidixic acid	S	R	S
14.	Piperacillin	S	S	S
15.	Streptomycin	R	R	R
16.	Tetracycline	R	S	R

S: Sensitive; R: Resistant; I: Intermediate.

Table 4. Minimum inhibition concentration (MIC) of different metal ions for isolated bacterial strains (RMB1, RMB2 and RMB6) from tannery wastewater.

Sr. No.	Metal ions used in study	Minimum inhibition concentration (MIC) (µg ml^{-1}) of metal ions for isolated bacterial strains		
		RMB1	RMB2	RMB6
1.	Cd	160	180	170
2.	Cr	500	450	480
3.	Cu	250	280	250
4.	Ni	500	530	520
5.	Mn	480	450	500
6.	Pb	380	290	300
7.	Zn	600	580	540
8.	Fe	700	590	610

teratogenic agents.

CONCLUSIONS

The contamination of soil and aquatic environment with industrial wastewaters containing toxic metals and a variety of organic pollutants could be a major source of multi-drug and multi-metal resistant pathogenic microbes. The study also confirmed that the isolated bacterial strains were *Pantoea* sp*., E. coli sp*., and *Lysinibacillus* spp*.* having multi-drug and multi-metal resistant property. It emerges that wastewater treatment processes should be modified significantly to assure their environmental safety.

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REFERENCES

Abideen S, **Babuselvam** M, **2014**. Antagonistic activity of *Lysinibacillus fusiformis* n 139 strain isolated from marine fish *Triacanthus strigilifer* and genome sequence Int J Curr Microbiol App Sci, 3(4): 1066-1072.

APHA, **2012**. Standard Methods for Examination of Water and Waste Water. 22nd edition. American Public Health Association, American Water Works Association Water Environment Federation. Washington, DC.

Barrow GI, **Feltham** RKA, **1993**. Cowan and steel's manual for the identification of medical bacteria, 3rd edn. Cambridge University

Press, Cambridge.
Bharagava RN, Chandra R, Rai V, **2009**. Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoidins from distillery effluent. World J Microbiol Biotechnol, 25: 737-744.
Bharagava RN, Yadav S, Chandra R, **2014**. Antibiotic and heavy metal resistance properties of bacteria isolated from the aeration lagoons of common effluent treatment plant (CETP) of tannery industries (Unnao, India). Ind J Biotechnol, 13: 514-519.
Chandra R, Bharagava RN, Kapley A, Purohit HJ, **2011**. Bacterial diversity, organic pollutants and their metabolites in two aeration lagoons of common effluent treatment plant (CETP) during the degradation and detoxification of tannery wastewater. Bioresour Technol, 102: 2333-2341.
Chandra R, Bharagava RN, Yadav S, Mohan D, **2009**. Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. J Hazard Mater, 162: 1514–1521.
Coelho MRR, Weid IVD, Zahner V, Seldin L, **2003**. Characterization of nitrogen- fixing *Paenibacillus* species by polymerase chain reaction-restriction fragment length polymorphism analysis of part of genes encoding 16S rRNA and 23S rRNA and by multilocus enzyme electrophoresis. FEMS Microbiol Lett, 222: 243-250.
DIFCO, **1984**. Laboratories Inc, Difco Manual, 10th ed. DIFCO Laboratories Inc. Detroit, Michigan.
Durai G, **Rajasimman** M, **2011**. Biological treatment of tannery wastewater - A review. J Environ Sci Technol, 4(1): 1-17.
El-Lathy MA, El-Taweel GE, El-Sonosy WM, Samhan FA, Moussa TAA, **2009**. Determination of pathogenic bacteria in wastewater using conventional and PCR techniques. Environ Biotechnol, 5(2): 73-80.
Flores PEO, Miranda GM, Romero FS, Mendoza MA, Sandoval TH, Silva RHV, **2012**. Pantoea agglomerans in immunodeficient patients with different respiratory symptoms. Sci World J, 1-8.
Jain PK, Ramchandran S, Shukla V, Bhakuni D, Verma SK, **2009**. Characterization of metal and antibiotic resistance in a bacterial population isolated from a copper mining industry. Int J Integrat Biol, 6: 57-61.
Kongjao S, Damronglerd S, Hunsom M, **2008**. Simultaneous removal of organic and inorganic pollutants in tannery wastewater using electrocoagulation technique. Korean J Chem Eng, 25: 703-709.
Lofrano G, Meric S, Zengin GE, Orhon D, **2013**. Chemical and biological treatment technologies for leather tannery chemicals and wastewaters: A review. Sci Total Environ, 461-462: 265-281.
Ma Q, Qu Y, Shen W, Zhang Z, Wang J, Liu Z, Li D, Li H, Zhou J, **2015**. Bacterial community compositions of coking wastewater treatment plants in steel industry revealed by Illumina high-throughput sequencing. Bioresour Technol, 179: 436-443.
Malik A, **Jaiswal** R, **2000**. Metal resistance in Pseudomonas strains isolated from soil treated with industrial wastewater. World J Microbiol Biotechnol, 16: 177-182.
Nithya R, **Sudha** PN, **2016**. Removal of heavy metals from tannery effluent using chitosan-g-poly(butyl acrylate)/bentonite nanocomposite as an adsorbent. Textiles Clothing Sustain, 2: 7.
Ozgunay H, Colak S, Mutlu MM, Akyuz F, **2007**. Characterization of leather industry wastes, Polish J Environ Study, 16, 867-873.
Paul BT, Clement GY, Anita KP, Dwayne JS, **2012**. Heavy metals toxicity and the environment. EXS, 10: 133-164.
Ramteke PW, Awasthi S, Srinath T, Joseph B, **2010**. Efficiency assessment of Common Effluent Treatment Plant (CETP) treating tannery effluents. Environ Monitor Assessment, 169: 125-131.
Singh C, Chowdhary P, Singh JS, Chandra R, **2016**. Pulp and paper mill wastewater and coliform as health hazards: A review. Microbiol Res Int, 4(3):28-39.
Tamura K, Dudley J, Nei M, Kumar S, **2007**. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecul Biol Evolut, 24: 1596-1599.
Viti C, Pace A, Giovannetti L, **2003**. Characterization of Cr(VI)-resistant bacteria isolated from chromium-contaminated soil by tannery activity. Curr Microbiol, 46: 1-5.
Wang Z, Zhang XX, Lu X, Liu B, Li Y, Long C, Li A, **2014**. Abundance and diversity of bacterial nitrifiers and denitrifiers and their functional genes in tannery wastewater treatment plants revealed by high-throughput sequencing. PLoS One, 9(11): e113603.
Yadav A, Raj A, Bharagava RN, **2016**. Detection and characterization of a multi-drug and multi-metal resistant enterobacterium *Pantoea* sp. from tannery wastewater after secondary treatment process. Int J Environ Bot, 1(2): 37-42.

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