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Prevalence of Bacterial Isolates in Tannery Effluent Collected from Vellore District, Tamil Nadu

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Abstract: Tanning industry contributes significantly towards exports, employment generation and occupies an important role in Indian economy. On the other hand, tannery wastes are ranked as the highest pollutants among all the industrial wastes. The present research deals with isolation and identification of various bacterial species from Tannery effluent and analysis of Physico-chemical characteristics of tannery effluent. Ten bacterial strains were isolated on the basis of morphological and biochemical analysis revealed that the isolates were *Pseudomonas fluorescens*, *Proteus* sp., *Bacillus* sp., *Escherichia coli, Serratia* sp., *Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter asburiae*, *Alcaligenes* sp. and *Micrococcus* sp. The effluent contain highest amount of BOD, COD and various toxic chemicals including heavy metals. It was sure that the tannery effluent which was excreted to our environment causes pollution.

Key words: Tannery effluent · Physico-chemical characteristics · Heavy metals and Bacteria

INTRODUCTION

Global environmental regulation is challenging the leather processing industry. The process of tanning involves the use of large amounts of fresh water and various chemical. Various chemicals used in tanning are lime, sodium bicarbonate, common salt, sodium sulphate, chrome sulphate, fat liquors, vegetable oils and dyes [1]. Tannery contains a higher amount of metals especially chromium, copper and cadmium. These effluents released on the land as well as dumped in to the surface water which ultimately leaches to ground water and lead to contamination due to accumulation of toxic metallic components and resulted in a series of well documented problems in living beings because they cannot be completely degraded. Hence, industrial effluents offer a wide scope of environmental problems and health hazards are becoming more complex and critical not only in developing countries like India but also in developed countries. Hence, the tannery waste is always characterized by its strong colour (reddish dull brown), high BOD, high pH and high dissolved solids [2].

MATERIALS AND METHODS

Collection of Tannery Effluent Samples: The tannery effluent to be bioremediated was collected from Vaaniyambadi, Vellore district of Tamil Nadu, India.

Isolation of Bacteria from Tannery Effluent: Pour plate technique was used for the isolation of bacteria from the tannery effluent collected from Vaaniyambadi, Vellore district, Tamil Nadu, India. In this method, 1 ml of sample was thoroughly mixed with 99 ml of sterile distilled water and then it was serially diluted by following standard procedure upto concentration of 10^{-6}. Then, 1 ml of serially diluted samples from each concentration of samples were transferred to sterile petriplates and evenly

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distributed throughout the plates and sterile unsolidified Nutrient agar was poured and it was allowed to solidify. The Nutrient agar plates were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated from the plates.

Identification of the Bacterial Isolates: Identification of the bacterial isolates was carried out by the routine bacteriological methods *i.e.,* discribed by Buchanan and Gibbons [3].

- By the colony morphology [4]
- Preliminary tests like Gram staining [5], Capsule staining [6], Endospore staining [7], Motility [8], Catalase [9] and Oxidase [10].
- Plating on selective medium [11].
- By performing biochemical tests [12].

Microscopic and biochemical tests were applied to this isolate according to Bergey's manual of systematic bacteriology. The genus to which the isolates belong were determined.

Analysis of Physico – Chemical Characteristics of Collected Tannery Effluent

Physical Parameters

Colour: The colour of the collected tannery was observed visually.

Odour: The odour of the collected tannery effluent was categorized as pleasant or unpleasant by direct smelling of the sample.

Temperature: The temperature of the tannery effluent was noted using Thermometric method at the site of sampling using portable calibrated mercury thermometer [13].

pH: The pH of the tannery effluent was determined by Potentiometric method using pH meter already standardized by using buffer solutions of known value before analysis.

Electrical Conductivity (EC): Electrical conductivity is the measure by conductivity meter following the procedure of Richard [14].

Total Suspended Solids (TSS): The TSS was determined by using the following formula which is given below [15].

TSS mg/L = (Final wt - Initial wt)/Amount of sample taken × 1000

Total Dissolved Solids (TDS): Total dissolved solids (TDS) are the measured by Richard (1954) by using Electrical Conductivity (EC) meter.

TDS (mg/L) = EC ìs/cm × 0.67

Total Hardness: Total Hardness was determined by following the below given formula.

Total Hardness (mg $CaCO_3$/L) = A × B × 1000/ ml sample

where, A = ml EDTA titrated for sample, B = mg $CaCO_3$ equivalent to 1 ml EDTA titrant

Estimation of Biological Oxygen Demand (BOD): Estimation of BOD was done by Winklers iodometric method [16].

Calculation for DO:

Volume of 0.025 N sodium thiosulphate used in the titration = DO in mg/L, DO at 0°C 760 mm pressure = DO × 0.07 mg/L

Calculation for BOD:

BOD (5 days at 20°C) = (DO_0 - DO_5 - BC) × 100 per cent sample.

DO_0 = Initial DO, DO_5 = DO after 20°C incubation for 5 days, BC = Blank correction i.e., difference in DO of blank on the initial day and after 5 days incubation.

Estimation of Chemical Oxygen Demand (COD): Estimation of COD was carried out by Titrimetric method. Calculation:

COD in mg/L = (Blank titre value - Sample titre value) × 0.125 × 1000 × 8 volume of the sample taken.

Chemical Parameters: Analysis of chemical parameters of tannery effluent was done by using Trimetric analysis. Briefly, Total hardness as $CaCO_3$ (Ca and Mg) was determined by titration of samples against EDTA and Chloride against $AgNO_3$.

Estimation of Calcium and Magnesium: Estimation of Calcium and Magnesium by EDTA Titrimetric method.

$$\text{Calcium and Magnesium in mg/L} = \frac{\text{Vol of 0.02N EDTA (consumed in Ca + Mg titration)} \times N \times 500 \times 100}{10 \times 10}$$

Estimation of Chloride: Estimation of Chloride by Silver nitrate Titrimetric method [17]. If the silver nitrate solution is exactly 0.0282 N,

Chloride mg/L = Volume of 0.0282 N consumed (sample - blank) × 1000

Estimation of Sodium and Potassium: Estimation of Sodium and Potassium was carried out by Flame photometric method.

Estimation of Sulphate: Estimation of Sulphate was done by Turbidimetric method.

Trace Metal Analysis: The estimation of trace heavy metals such as for Cr, Zn, Cu, Pb, Ni in the industrial effluent was performed as per Malik *et al*. [18].

RESULTS AND DISCUSSION

Identification of Bacteria Isolated from Tannery Effluent: In the collected tannery effluent, ten different bacteria were identified and the characteristics of the bacterial isolates were furnished in Table – 1 and Table - 2. The identified bacteria strains were designated as TEB - 1 to TEB - 10. The identified bacterial isolates were *Pseudomonas fluorescens*, *Proteus* sp., *Bacillus* sp., *Escherichia coli, Serratia* sp., *Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter asburiae*, *Alcaligenes* sp. and *Micrococcus* sp.

Metal resistance determinants were initially found on bacterial plasmids. *Escherichia coli* and *Alcaligenes* have possession of a heavy metal resistance. Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell and reduction of the heavy metal ions to a less toxic state [19, 20].

Analysis of Physico - Chemical properties of Tannery effluent: The physico – chemical characteristics of the tannery effluent collected from Vaniyambadi region, Vellore district, Tamil Nadu, India was analyzed and the results were furnished in Table – 3. The tannery effluent was acidic in nature with brown colour and emitted unpleasant smell. The temperature of collected tannery effluent was 38°C and the EC was 30.2 dSm^{-1}. The hardness of the tannery effluent was recorded as 5200 mg/L. The Total Suspended Solid (TSS) and Total Dissolved Solid (TDS) present in collected tannery effluent were 316 mg/L and 1500 mg/L respectively. It also showed high value of Biological oxygen demand (BOD) (1280 mg/L) and Chemical oxygen demand (COD) (2037 mg/L). High amount of chloride (1464 mg/L), calcium (160 mg/L), magnesium (57 mg/L), sodium (188 mg/L), potassium (603 mg/L), fluoride (6.0 mg/L), nitrate (45 mg/L), nitrite(33 mg/L), sulphate (349 mg/L) and the toxic heavy metals (Cr^{2+} - 146 mg/L, Ni^{2+} - 57 mg/L, Zn^{2+} - 36 mg/L, Cu^{2+} - 29 mg/L, Fe^{2+} - 19.00 mg/ L, Cd^{2+} - 54 mg/L, Pb^{2+} - 4.6 mg/L and Mn^{2+} - 9.9 mg/L) were recorded in the collected tannery effluent sample.

Table 1: Preliminary characteristics of bacteria isolated from tannery effluent

Isolate No.	Gram staining	Shape	Spores	Motility	Nutrient agar	Mac Conkey agar	EMB Agar	MSA Agar	*Pseudomonas* isolation agar	Growth at 42°C
TEB– 1	-	Rods	-	-	Red pigmented colonies	LF	-	-	-	-
TEB – 2	-	Rods	-	+	Swarming motility and fishy odour	NLF	-	-	-	-
TEB – 3	+	Rods	+	-	Large, circular and white colonies	NLF	-	-	-	-
TEB -4	-	Rods	-	+	Circular and colourless colonies	LF	Green metallic sheen observed	-	-	-
TEB -5	+	Cocci	-	-	Smooth, golden yellow colonies	LF	-	Golden yellow colonies	-	-
TEB -6	-	Rods	-	+	Bluish green colonies	NLF	-	-	Bluish green colonies	+
TEB -7	-	Rods	-	+	Fluorescent pigmented colonies	NLF	-	-	No bluish green colonies	-
TEB -8	-	Rods	-	-	Mucoid and cream colonies	NLF	-	-	-	-
TEB -9										
TEB -10										

TEB –Tannery effluent bacteria; + - Positive; - - Negative; NLF – Non lactose fermenting colonies; LF – Lactose fermenting colon

Table 2: Biochemical characteristics of bacteria isolated from Tannery effluent

	Sugar fermentation (Acid/Gas)											
Isolate No.	Glucose	Sucrose	Mannitol	Mannose	Arabinose	Dextrose	Catalase	Oxidase	Gelatinase	Caseinase	Starch	Nitrate reduction
TEB – 1	+/-	+/-	+/-	+/-	-	-	+	-	+	+	-	-
TEB – 2	+/+	-	-	-	-	-	+	-	-	-	-	-
TEB – 3	+/-	-	+/-	-	-	-	+	-	+	-	+	+
TEB -4	+/+	+/+	+/+	-	-	-	+	-	-	-	-	-
TEB -5	+/-	+/-	+/-	-	-	+/-	-	-	-	-	-	-
TEB -6	+/+	-	-	-	-	-	+	+	+	+	-	-
TEB -7	+/-	-	+/-	+/-	+/-	-	+	+	+	+	-	-
TEB -8	+/-	+/-	+/-	-	-	-	+	-	-	-	-	-
TEB -9	+/-	+/-	+/-	-	-	+/-	-	-	-	-	-	-
TEB -10	+/-	-	+/-	-	-	-	+	-	+	-	+	+

Table Continued

	Urease	Indole	MR	VP	Citrate	TSI	Lipase	Pectinase	DNase	Coagulase	Phenylalanine deaminase test
TEB – 1	-	+	+	+	+	-	-	-	-	-	-
TEB – 2	+	-	-	-	+	A/K, H_2S^+, G^-	-	-	-	-	+
TEB – 3	-	-	-	+	+	-	-	-	-	-	-
TEB – 4	-	+	+	-	-	A/A, H_2S^-, G^+	-	-	-	-	-
TEB – 5	-	-	-	+	+	-	-	-	+	+	-
TEB – 6	+	-	-	-	+	K/K, H_2S^-, G^-	+	-	-	-	-
TEB – 7	+	-	-	-	+	-	-	+	-	-	-
TEB – 8	+	-	+	-	+	-	-	-	-	-	-
TEB – 9	-	-	-	+	+	-	-	-	+	+	-
TEB – 10	-	-	-	+	+	-	-	-	-	-	-

TEB –Tannery effluent bacteria; + - Positive; - - Negative; +/+ - Acid/Gas; +/- - Acid/ No gas; A/A – Acid butt and alkali slant; A/A – Acid butt and acid slant; K/K – Alkali butt and Alkali slant; H_2S^- - No Hydrogen sulphide production; H_2S^+ - Hydrogen sulphide production; G^+- Gas produced; G^- - Gas not produced; *TEB–1 Serratia* sp; TEB-2 *Proteus* sp.; TEB-3 *–Bacillus subtilis* ; TEB-4 *Escherichia coli* ; TEB-5 *–Staphylococcus aureus*.; TEB-6 – *Pseudomonas aeruginosa* ; TEB-7 – *Pseudomonas fluorescens*, TEB-8 – *Enterobacter asburiae*, TEB-9 *–Alcaligenes* sp.; TEB-10 *–Micrococcus* sp.

Table 3: Physico-chemical properties of collected Tannery effluent

Parameters	Raw Effluent	BIS Limit Is 2490-2009
Colour	Brown	-
Odour	Offensive	-
Temperature	38°C	-
pH	9.7	5.5 - 9.0
Electrical conductivity (dsm^{-1})	30.2	NM
Total hardness (mg/L)	5200	100
Total suspended solids (mg/L)	316	100
Total dissolved solids (mg/L)	1500	2100
Biological Oxygen Demand (mg/L)	1280	30
Chemical Oxygen Demand (mg/L)	2037	250
Carbonate (mg/L)	860	600
Bicarbonate (mg/L)	1428	NM
Calcium (mg/L)	160	200
Magnesium (mg/L)	57	30-100
Chloride (mg/L)	1464	1000
Sodium (mg/L)	188	NM
Potassium (mg/L)	603	NM
Fluoride (mg/L)	6.0	2.0
Nitrate (mg/L)	45	100
Nitrite (mg/L)	33	10
Sulphate (mg/L)	349	1000
Chromium (mg/L)	146	0.5
Nickel(mg/L)	57	3.0
Zinc (mg/L)	36	-
Copper(mg/L)	29	3.0
Iron (mg/L)	19	-
Cadminum (mg/L)	5.4	2.0
Lead (mg/L)	4.6	0.1
Manganese (mg/L)	9.9	-

TNPCB- Tamil Nadu pollution board

The effluent from industries can reduce the pressure on water scarcity for irrigation as well as the effluents contaminated the water bodies increased the water scarcity [21]. The use of excessive amount of chemicals used in leather processing gives high concentrations of discharged heavy metals. Chromium is known to cause of cancer. The recommended limit for maximum amount of chromium in the effluent samples is 1.0 mg/L [22, 23].

CONCLUSION

The industrial effluents are enriched media of microbial population it's resistant to heavy metals present in effluent. The microbial species used as a bioremediation tool for the treatment of effluent. The physico-chemical characters of tannery effluent were concluded all the parameters were higher than the Tamil Nadu Pollution board not recommended for Agriculture.

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