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Research Article

Isolation and Identification of Bacterial Strains from Tannery Effluent and its Capability Assessment to Degrade Leather Dye

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ABSTRACT

This study focused on the isolation and identification of potential dye degrading bacteria from tannery effluent. The ability of these bacteria to decolorize three leather dyes *viz*. black, brown and red dyes were also examined. Based on morphological, cultural, physiological and biochemical properties, isolates were provisionally identified up to species level as *Pseudomonas putida* (RS1A), *Bacillus sphaericus* (RS1B), *Bacillus brevis* (RS1C), *Pseudomonas stutzeri* (RS1D), *Alcaligenes eutrophus* (RS1E), *Listeria denitrificans* (RS2A), *Listeria grayi* (RS2B), *Listeria murrayi* (RS2C), *Bacillus coagulans* (RS2D) and *Listeria monocytogenes* (RS2E). In dye decolorization study, *Pseudomonas stutzeri* (RS1D) completely decolorized 1000 ppm black dye within 8 days. *Listeria monocytogenes* (RS2E) was found most potential strain capable of completely decolorizing up to 1000 ppm of black dye and same conc. of brown dye moderately during 9 and 10 days of incubation, respectively. In contrast, weak decolorization of red dye was observed by *Bacillus brevis* (RS1C), Pseudomonas stutzeri (RS1D) and *Listeria monocytogenes* (RS2E) at low conc. e.g., 100-200 ppm. *Bacillus coagulans* (RS2D) was found least potential in decolorizing all dyes. Irrespective of dyes, *Listeria monocytogenes* (RS2E) was the most potent and its potentiality can be exploited to clean up the environment.

Keywords: Bacteria; Decolorization; Dye; Tannery effluent

INTRODUCTION

Environmental pollution is recognized as one of the major problem of the modern world [1]. Major essential elements of life such as air, water and soil are being contaminated constantly due to increasing industrialization and rapid urbanization [2]. This is because different industries apply approximately 10,000 different types of dye and these dye-based pollutants initiate a series of processes, affecting both its biotic and abiotic elements, once they enter into an ecosystem [3]. Color in the effluent is an obvious indicator of water pollution and the discharge of highly colour synthetic dye effluents can damage the receiving water body by impeding penetration of light. Moreover, dyes as well as their breakdown products can be cytotoxic or carcinogenic [4]. On a daily basis, more than 16,000 m3 of highly toxic effluents with a BOD (biochemical oxygen demand) load of 17,600 kg per day are being disposed by the tanneries in Bangladesh. An estimated 0.35 t per day of chromium is discharged into a lagoon of 25 ha. Studies showed that the subsoil of Hazaribagh tannery area is seriously contaminated with Cr, Zn, Cu and Pb in addition to phenols and hydrocarbons [5-7]. Several physicochemical methods, such as adsorption, coagulation, precipitation, filtration and oxidation have been used to treat dyestuff effluents, but these methods have many limitations and disadvantages. All of the advanced recovery techniques cost heaps of money, effort, and time which are not affordable by many developing nations. The cost effective choice to halt this process is decontamination through microbial functions. The microorganisms can use the chemical substances as sources of energy and nutrients in soils, which are an important sink for many pollutants. In addition to utilizing natural substances, microorganisms have an extensive capacity to degrade synthetic substances [8]. It is therefore, important to develop efficient and cost-effective methods for the decolorization and degradation of dyes in industrial effluents and contaminated soil [9]. The isolation and identification of synthetic dye associated bacteria is significant

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for their dye degradation capability. Marzan et al. [10] studied heavy metal resistance and biodegradation capacity of local strains as bioremediation agents in toxic tannery effluent treatment technology. They isolated three bacteria capable of detoxifying heavy metals (Chromium, Cadmium and Lead) from tannery effluent in Chittagong, Bangladesh. In similar approach, effluent treatment plant of Madina tannery was selected which is located near Oxygen point in Chittagong, Bangladesh. Large residential areas have been observed in the surroundings of Madina tannery. So, this study aimed to find out bacteria that occur naturally in the tannery effluent and hold the capability to degrade very complex synthetic dyes in natural conditions.

MATERIALS AND METHODS

Tannery effluent samples were collected in pre-sterilized 500 ml screw cap bottles from two different sites of Modina tannery effluent treatment plant. Samples were immediately transported to the laboratory and were preserved at 4°C before analysis and during experiments. Temperature and pH were tested immediately using thermometer and pH meter respectively in the laboratory. Chromium and Chlorine content were also tested using Multi parameter Bench Photometer. BOD and COD were determined following APHA [11]. Microbial load in samples was determined by pour plate method. Samples were diluted upto 10-3 and then plated in sterile petri plates. Pre-sterilized Nutrient Agar (NA) was poured to petri plates and after incubation at 37°C for 24 h; number of developed colonies were counted.

Isolation and identification of bacteria

Discrete colonies appeared after 24 h of incubation were selected based on colony morphology such as color, form, elevation, margin and surface etc. from dilution plates. Selected colonies were picked up and streaked on Nutrient Agar plate to get pure culture. Pure cultures thus obtained were transferred to agar slant for further study. Isolates from two different samples were coded as RS1A, RS1B, RS1C, RS1D, RS1E, RS2A, RS2B, RS2C, RS2D and RS2E. Bacterial isolates were identified based on morphological, physiological and biochemical characteristics. All the characteristics were compared with the standard description in the Bergey's Manual of Determinative Bacteriology, 8th edition (Buchanan and Gibbons, 1974). The tests carried out were simple staining, motility test, Gram staining, spore staining, acid fast staining, Catalase test, Oxidase test, Urease test, IMViC, nitrate reduction, H2S production, casein hydrolysis, starch hydrolysis, gelatin hydrolysis, deep glucose agar test, growth response at different temperature, pH, salt concentration, growth in synthetic media and inorganic salt, fermentation of glucose, lactose, xylose, galactose, maltose, sorbose, raffinose, mannitol, rhamnose, arabinose, mannose, sucrose, fructose and soluble starch.

Decolorization study of isolates

All the isolates were challenged with red, black and brown dye commonly used as leather dye in tannery industry. For this purpose, nutrient broth containing five different concentrations e.g., 100, 200, 1000, 1500 and 2000 ppm of previously mentioned dyes were prepared, dispensed 10 ml of media in each test tube and sterilized. Tubes were inoculated with selected individual isolates and incubated at previously determined optimum temperature for up to 10 days. Optimum temperature for each isolates. Tubes were observed periodically to check decolorization of modified media. At the same time an uninoculated test tube containing sterile media with each concentration of dye was kept as control.

RESULTS AND DISCUSSION

Identification of isolates

A total of 29 different types of colonies were isolated and purified. Out of 29 isolates, 10 isolates were selected and identified based on morphological, cultural, physiological and biochemical properties. Colony morphology and growth pattern of each isolates were observed as given in Table 2. Results of morphological, staining and physiological characteristics of isolates are given in Table 3. Among the isolates, RS1A, RS1D and RS1E were gram negative and the rests were gram positive. All isolates were motile and non-acid fast. Isolate Nos. RS1B, RS1C and RS2D were spore former and others were non-spore former (Table 3). Table 4 shows biochemical properties and fermentation results of these isolates. All isolates are Catalase positive and Indole negative. All isolates except RS1C

Table 1: Physicochemical characteristics and total viable count of the collected samples.

No. of Sample	Color of sample	Temp. (°C)	pH	BOD (mg/L)	COD (mg/L)	Chromium (µg/L)	Chlorine (mg/L)	Total Viable Count (cfu/ml)
1	bluish black	29	6.3	8.7	0.211	7.0	0.25	76×10^3
2	Ash	27	10.2	10.2	0.320	3.7	0.11	37×10^3

Table 2: Colony morphology and growth pattern of isolates in different media.

Isolate Nos.	Colony morphology					Growth pattern in		
	Color	Form	Elevation	Margin	Surface	Agar slant	Nutrient Broth	Glucose broth
RS1A	Fluorescent	Circular	Raised	Entire	Smooth	Echinulate	No sediment	No sediment
RS1B	Translucent	Irregular	Raised	Erose	Smooth	Spreading	Sediment	Sediment
RS1C	Translucent	Irregular	Raised	Undulate	Wrinkle	Spreading	Sediment	Sediment
RS1D	Off white	Circular	Convex	Entire	Smooth	Echinulate	Sediment	No sediment
RS1E	Milk white	Circular	Flat	Entire	Smooth	Echinulate	No Sediment	No sediment
RS2A	Translucent	Irregular	Convex	Entire	Smooth	Filiform	No sediment	No sediment
RS2B	Bluish Gray	Circular	Convex	Entire	Wrinkle	Filiform	Sediment	Sediment
RS2C	Bluish Gray	Circular	Convex	Entire	Radiate	Spreading	Sediment	Sediment
RS2D	Translucent	Circular	Raised	Entire	Smooth	Filiform	Sediment	Sediment
RS2E	Bluish Gray	Circular	Flat	Entire	Smooth	Spreading	Sediment	Sediment

Table 3: Morphological, staining and physiological characteristics of isolates.

Features / Tests	RS1A	RS1B	RS1C	RS1D	RS1E	RS2A	RS2B	RS2C	RS2D	RS2E
Shape	Short rod	Straight rod, Spherical	Rod	Rod	Oval rod	Filamentous rod	Short rod	Short rod	Short rod	Short rod
Arrangement	Single, pair, cluster	Single, short chain	Single	Single, pair, chain	Single, short chain	Single, pair, club shaped	Single, pair	Single, pair	Single	Single, pair
Motility	+	+	+	+	+	+	+	+	+	+
Gram Staining	-	+	+	-	-	+	+	+	+	+
Spore Staining	-	+	+	-	-	-	-	-	+	-
Acid fast Staining	-	-	-	-	-	-	-	-	-	-
O_2 Requirement	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic & Microaerophilic	Aerobic & Microaerophilic	Aerobic	Aerobic
Optimum pH	7	7.5	7.5	7	7	6.5	6.5	6.5	6	7
Opt. Temp (°C)	27	30	35	35	30	35	35	35	40	35
Growth in										
Synthetic media	-	+	+	+	+	+	+	+	+	-
Inorganic salt	-	-	-	+	-	-	-	-	-	-
Salt conc. (%)	1-4	1-4	1-2	1-4	2-4	3.5-4	1-3	4-6	1-6	2-8

Table 4: Biochemical properties& Fermentation study of isolates.

Test Name	RS1A	RS1B	RS1C	RS1D	RS1E	RS2A	RS2B	RS2C	RS2D	RS2E
Catalase test	+	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	-	-	-	-	+	-
Urease test	+	+	-	+	+	+	+	+	+	+
Indole test	-	-	-	-	-	-	-	-	-	-
H2S production	-	+	-	-	+	-	+	-	+	-
Casein hydrolysis	-	+	+	-	+	+	+	-	+	-
Starch hydrolysis	+	-	-	+	-	-	+	+	+	-
Albumin hydrolysis	-	-	+	+	-	-	-	-	-	+
Gelatin liquefaction	-	+	+	-	-	+	-	-	+	-
Citrate Utilization	+	-	+	+	-	+	+	+	-	+
Nitrate Reduction	+	-	+	+	+	+	-	+	-	-
VP Test	-	-	-	+	-	-	+	+	+	-
MR Test	-	-	-	+	-	-	+	-	-	+
Glucose fermentation	+	-	+	+	+	+	+	+	-	+
Lactose fermentation	-	-	-	+	-	+	+	-	+	+
Xylose fermentation	+	-	-	-	-	+	-	-	+	-
Galactose fermentation	+	-	-	-	+	-	+	+	+	-
Maltose fermentation	-	-	-	+	+	+	+	+	+	+
Sorbose fermentation	-	-	-	-	-	-	-	-	+	-
Raffinose fermentation	-	-	-	-	-	+	+	-	+	-
Mannitol fermentation	-	-	+	+	-	+	+	+	+	-
Rhamnose fermentation	-	-	-	+	+	+	-	+	+	+
Arabinose fermentation	-	-	-	+	-	+	+	-	+	-
Manose fermentation	-	-	-	-	-	-	-	-	-	-
Sucrose fermentation	+	-	+	+	+	+	+	+	+	+
Fructose fermentation	+	-	-	-	+	+	+	+	+	
Soluble starch fermentation	-	-	-	-	-	-	+	+	+	+

were Urease positive. Out of 10 isolates, 5 isolate *viz*. RS1A, RS1C, RS1D, RS1E and RS2D were Oxidase positive. Among the isolates 4 were H2S producer and others are non-producer. Only 6 isolates could hydrolyze casein, others could not. 5 isolates showed the ability to hydrolyze starch and others do not. Three out of ten isolates were able to hydrolyze albumin. Only 3 isolates show gelatin hydrolyzing capability and others do not. Other than RS1B, RS1E and RS2D, all isolates could utilize citrate. Six isolates could reduce nitrate, 3 isolates were Methyl-Red (MR) positive, 4 isolates showed positive reaction in VP test and others were negative. Comparing morphological, cultural, physiological and biochemical properties of isolates with standard description in Bergey's Manual of

Determinative Bacteriology, isolates were provisionally identified as *Pseudomonas putida* (RS1A), *Bacillus sphaericus* (RS1B), *Bacillus brevis* (RS1C), *Pseudomonas stutzeri* (RS1D), *Alcaligenes eutrophus* (RS1E), *Listeria denitrificans* (RS2A), *Listeria grayi* (RS2B), *Listeria murrayi* (RS2C), *Bacillus coagulans* (RS2D), *Listeria monocytogenes* (RS2E). From textile effluent, Mahbub et al. [12] isolated four species of *Bacillus viz. Bacillus fastidious*, *Bacillus polymyxa*, *Bacillus licheniformis*, *Bacillus megaterium*, and *Staphylococcus aureus* and *Micrococcus luteus*. Sharnaik and Kaneker [13] reported decolorization potential of *Pseudomonas* isolated from textile dyeing industry. Ozdemir et al. [14] studied decolorization and COD removal of leather industry dyes and isolated three different strains *viz. Aeromonas hydrophila*, *Proteus vulgaris* and *Providencia rettgeri* capable of decolorizing azo dyes.

Dye decolorization studies

Results of dye decolorization pattern of each isolate on each dye are given in Table 5. *Pseudomonas stutzeri* (RS1D) was found most effective in decolorizing black dye and this isolate could completely decolorize 100 ppm of this dye within 5 days and took only 8 days to decolorize upto 1000 ppm completely when it was incubated at pH 7.0 and 35°C. But decolorizing capability of *Pseudomonas stutzeri* (RS1D) significantly reduced with increasing concentration above 1000 ppm and found to decolorize weakly at 1500 ppm. *Listeria monocytogenes* (RS2E) took 4, 6 and 9 days to decolorize the challenged black dye at concentration of 100 ppm, 200 ppm and 1000 ppm, respectively at pH 7.0 and 35°C. *Alcaligenes eutrophus* (RS1E) and *Bacillus coagulans* (RS2D) were found least effective in decolorizng black dye. *Psedomonas putida* (RS1A), *Bacillus brevis* (RS1C) could efficiently decolorize the dye upto 200 ppm. Considering days taken to decolorize and dye concentration up to which isolates can decolorize *Pseudomonas stutzeri* (RS1D) and *Listeria monocytogenes* (RS2E) can be considered as most effective to decolorize black dye. At concentration of 100 ppm, *Listeria grayi* (RS2B) could decolorize brown dye most effectively in 5 days at pH 6.5 and 35°C, whereas *Bacillus brevis* (RS1C) and *Listeria monocytogenes* (RS2E) took 7 and 8 days respectively to decolorize this dye at same concentration. Similarly, *Pseudomonas stutzeri* (RS1D) and *Listeria denitroficans* (RS2A) were found to decolorize completely of brown dye within 7 days at 100 ppm at their optimum growth condition (Table 3) but at 1000 ppm they were found inactive. *Bacillus coagulans* (RS2D) was unable to decolorize brown dye even at 100 ppm. Depending on decolorization rate and dye concentration up to which isolates can decolorize, *Bacillus brevis* (RS1C) and *Listeria monocytogenes* (RS2E) are most potential for decolorizing brown dye. All isolates were less effective against red dye compared to other dyes. However, *Bacillus sphaericus* (RS1B), *Bacillus brevis* (RS1C), *Pseudomonas stutzeri* (RS1D), *Listeria murrayi* (RS2C) and *Listeria monocytogenes* (RS2E) could moderately decolorize red dye upto 100 ppm. *Bacillus brevis* (RS1C), *Pseudomonas stutzeri* (RS1D) and *Listeria monocytogenes* (RS2E) were found to decolorize red dye at 200 ppm. In this study, *Pseudomonas putida* (RS1A) and *Bacillus coagulans* (RS2D) were found to show least capability to decolorize red dye. In similar studies, decolorization of dye compounds by *Pseudomonas*, *Bacillus* and *Clostridium* were reported by Mihir et al. [15]. Sukumar et al. reported Bacillus sp. as maximum color reductive bacteria [16]. Leena and Selva Raj (2008) stated that effluent adapted bacteria are better candidates for decolorizing the effluent [17]. In another approach, Kumar et al. (1999) reported microbial tannase-catalyzed biotransformation of tannic acid by *Citrobacter freundii* isolated from tannery effluent [18]. Decolorization and degradation of the reactive dye by *Citrobacter sp.* CK3 was also reported by Hui et al. [19]. Our findings are in accordance with the abundance of *Bacillus*, *Pseudomonas*, *Alcaligenes* and *Listeria sp.* from tannery effluent.

CONCLUSION

In this study, bacteria isolated from tannery effluent treatment plant were provisionally identified based on morphological, cultural, biochemical and physiological characteristics. All isolates belonged to four genera such as *Pseudomonas*, *Bacillus, Alcaligenes* and *Listeria*. Potentiality of isolates was assessed by challenging it with three leather dyes. All the results presented in this study support that three strains such as *P. seudomonas stutzeri* (RS1D), *Listeria grayi* (RS2B) and *Listeria monocytogenes* (RS2E) have potentiality to decolorize tannery dyes and these can be used for the treatment of tannery effluent.

CONFLICT OF INTEREST

There is no conflict of interest in this research.

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Table 5: Decolorization pattern of black, brown and red dyes by isolates.

Name of Isolates	Black dye conc. (ppm)			Brown dye conc. (ppm)			Red dye conc. (ppm)		
	100	200	1000	100	200	1000	100	200	1000
RS_1A	+++ (7)	+++ (8)	+ (10)	+ (10)	- (10)	- (10)	- (10)	- (10)	- (10)
RS_1B	++ (6)	+ (7)	- (10)	++ (10)	+ (10)	- (10)	++ (10)	- (10)	- (10)
RS_1C	+++ (6)	+++ (9)	++ (10)	+++(7)	++ (8)	++ (10)	++(10)	+ (10)	- (10)
RS_1D	+++ (5)	+++ (6)	+++ (8)	+++ (7)	++ (8)	+ (10)	++ (10)	+ (10)	- (10)
RS_1E	- (10)	- (10)	- (10)	+ (9)	- (10)	- (10)	+ (10)	- (10)	- (10)
RS_2A	- (10)	- (10)	- (10)	+++ (7)	++ (10)	- (10)	+ (10)	- (10)	- (10)
RS_2B	++ (10)	- (10)	- (10)	+++ (5)	++ (10)	- (10)	+ (10)	- (10)	- (10)
RS_2C	++ (7)	+ (10)	- (10)	++ (9)	++ (10)	- (10)	++ (10)	- (10)	- (10)
RS_2D	- (10)	- (10)	- (10)	- (10)	- (10)	- (10)	- (10)	- (10)	- (10)
RS_2E	+++ (4)	+++ (6)	+++ (9)	+++ (8)	++ (10)	++ (10)	++ (10)	+ (10)	- (10)

Note: +++, ++ & + means complete, moderate & weak decolorization respectively. Numbers within parenthesis indicate days of incubation

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