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## Textile dye degradation using bacterial strains isolated from textile mill effluent

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### Abstract

Textile industry generates waste water with a complex mixture of many pollutants such as heavy metals, chlorinated compounds, pigments and dyes. The presence of textile dyes even in low concentration in effluent is highly visible, undesirable and recalcitrant in the natural environment. After release into the water bodies these dyes have negative impact on photosynthesis of aquatic plants. Bacterial strains capable of degrading textile dyes were isolated from effluents of hand looms. The soil sample from Kannan hand looms, Balaramapuram was used for the isolation of textile dye degrading organisms. Totally five bacterial strains were isolated and designated as TDP1, TDP2, TDG1, TDG2 and TDG3. The isolates were grown on nutrient agar plates with a textile dyes Provisional pink and Green PB (50mg/100ml). The morphological and biochemical characteristics of isolates were determined. The decolourising ability of the strains were detected using nutrient broth incorporated with dye and the OD reading was recorded by UV- Spectrophotometer at 592nm. More than 90% of Provisional pink was reduced and colour changes occurs within five days at a dye concentration of 50mg/l. The decolourising ability of the strains were detected using nutrient broth with different dye concentration (50mg, 100mg, 150mg, 200mg & 250mg) at different time intervals. All the five strains showed decolourising ability when incorporated in nutrient broth with dye. Among those strains TDP1 and TDG3 showed maximum ability to decolourise the textile dyes. Identification of the strain TDP1 and TDG3 was achieved by 16srRNA sequencing and was identified as *Bacillus* and *Aeromonas hydrophila* sp.

**Keywords:** Hand looms, Provisional pink, Recalcitrance, UV - spectrophotometer.

### Introduction

Dyes are released into the environment in industrial effluents from two major sources, the textile and the dyestuff industries. A necessary criterion for the use of these dyes is that they must be highly stable in light and during washing. They must also be resistant to microbial attack. Therefore, they are not readily degradable and are typically not removed from water by conventional wastewater treatment systems. While most dyes are not particularly toxic, they are considered to be a pollutant.

The first synthetic dye, mauvein, was discovered in 1856. Since then, over 1,00,000 dyes have been generated worldwide with an annual production of over  $7 \times 10^5$  metric tones. Synthetic dyes are widely used in textile, paper, food, cosmetics and pharmaceutical industries. The efficiency in dyeing processes has resulted in 10-15% of unused dyestuff entering the waste water directly. Color present in dye effluent gives a straight forward indication of water being polluted and discharge of this highly colored effluent can damage directly the receiving water. Further more, it is difficult to degrade the mixtures of the wastewater from textile industry by conventional biological treatment processes, because their ratio of BOD/COD is less than 0.3 [3]. In some cases, traditional biological procedures were combined with physical- or chemical-treatment processes to achieve better decolorization [17], but chemical or physical-chemical methods are generally costly, less efficient and of limited applicability and produce wastes which are difficult to dispose. As a viable alternative, biological processes have received increasing interest owing to their cost effectiveness, ability to produce less sludge, and environmental benignity [2]. Therefore, to develop a practical bioprocess for treating dye-containing wastewater is of great significance. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Over the past decades, many microorganisms are capable of degrading azo dyes [15], including bacteria, fungi [5,16], yeast [8], actinomycetes [18] and algae [4].

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Most azo dyes are reduced anaerobically to the corresponding amines with cleavage of azo bonds by bacterial azoreductase, but they are difficult to degrade aerobically [2]. Moreover, fungal ligninolytic enzyme system (lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase might also be involved in the biodegradation of dyes. Biodegradation of dyes carried out by several bacterial species such as *Bacillus subtilis* [6, 9], *Bacillus sphaericus* [15] and *Pseudomonas* sp [12].

In general, the wastewater from textile industry contains various dyes. To gain a widespread reception, the dye degrading bacteria should exhibit decolorizing ability for a wide range of dyes. This study aimed to isolate some bacterial strains, which possessed the ability to decolorize two kinds of dyes, including provisional pink and green PB dyes. A bacterium displaying the greatest decolorizing ability was identified by 16S r RNA sequencing. The efficiency of the dye degrading strains at different dye concentrations were also studied.

## Materials and Methods

### Sample collection

Water and soil samples collected from waste disposal site of Kannan hand looms, Balaramapuram during December 2012. Samples were collected in Pyrex borosilicate glass containers. The samples were immediately collected and stored at 4 °C prior to the analysis.

### Isolation of bacteria

Nutrient agar supplemented with textile dye (50mg/100ml), pH 7.0 was used for the isolation of bacteria. The samples were appropriately diluted with sterilized distilled water and 0.1ml was spread on the media. After incubation for 24-48 hours at 30 °C, the isolated colonies were selected and purified.

The isolates were identified on the basis of their morphological, biochemical and molecular characterisation according to the method given by Bergey's Manuals (1994).

### Morphological identification

Gram staining and motility was carried out to determine the morphology of the organisms.

### Biochemical characterization

The biochemical tests such as IMViC, TSI, Oxidase test, Catalase test, urease test, Carbohydrate fermentation test, Amylase activity, Protease activity and Gelatin hydrolysis was performed and the results were observed.

### Decolourization experiment

The dye degrading bacterial strains were grown for 24 hours at 30 °C in 250ml Erlenmeyer flask containing nutrient broth. After 24 hours, 50mg dye/100ml was added and incubated at 30 °C for 120 rpm on orbital shaker (Remi, Cis24BL). The aliquot (3ml) of the culture media was withdrawn at different time intervals, centrifuged at 5000rpm for 15 min. Decolourization was monitored by measuring the absorbance of culture supernatant at 592 nm in UV spectrophotometer (Elico dougle beamSL210).

The percentage decolourization was calculated as follows:

$$\% \text{ decolourization} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

### Decolourization at different dye concentrations

The dye degrading bacterial strains were grown for 24 hours at 30 °C in 250ml Erlenmeyer flasks containing nutrient broth. After 24 hours, nutrient medium was added with 50,100, 150, 200, 250mg l<sup>-1</sup> dye and incubated at 30 °C for 120 rpm on orbital shaker. The aliquot (3ml) of the culture media was withdrawn at different time intervals, centrifuged at 5000rpm for 15 min. Decolourization was monitored by measuring the absorbance of culture supernatant at 592 nm in UV spectrophotometer.

### Identification of effective dye degrading strain by 16S r RNA sequencing

Identification of the isolated effective dye degrading strains (TDP1 & TDG3) strain was performed by 16srRNA sequencing. Total genomic DNA was extracted from a 5ml late exponential phase cell culture using genomic DNA and was isolated from the culture provided using chromous genomic DNA isolation kit (RKT09). PCR amplification of the 16s rRNA genes was performed with an ABI2720 thermocycler (PE Applied biosystems) using the 16s forward primer:PF15' - AGAGTTTGATCATGGCTC -3'(E coli 8-25)16s reverse primer:PB25'- AGAGTTTGATCATGGCTC - 3' (E coli 1523-1540)

The amplified products were purified with QIA quick PCR purification columns (Qiagen) following the manufacturers protocol. 10µl aliquots of each template were used for overnight digestion with a combination of the two restriction endonucleases AluI and RsaI (chromous lab). The digested amplified products were then screened by RFLP fingerprinting patterns. For each cluster obtained, two templates were selected at random for sequencing kit. Analysis of the sequences obtained was performed using Seq Scape\_v5.2 software and SEQUENCE analysis was done using the protocol formulated by BDTv3-KB-Denovo-v5.2 and the reaction plate used for the study is Applied biosystems micro Amp optical 96-well reaction plate and BLAST programme was used to identify the percentage similarity and identification the bacterial strain.

## Results

### Isolation and identification

Five bacterial isolates with diverse morphology were selected and the strains were named as TDP1& TDP2(degraded provisional pink), TDG1, TDG2 and TDG3(degraded green pb).The 5 isolates were identified as *Bacillus* sp (TDP1), *Pseudomonas* sp (TDP2), *Bacillus* sp (TDG1), *Staphylococcus* sp (TDG2) and *Aeromonas hydrophila* (TDG3). Among the 5 strains TDP1 (*Bacillus* sp) and TDG3 (*Aeromonas hydrophila*) showed maximum decolourisation of dyes. Blast analysis showed that the isolate TDP1 showed 100% similarity with *Bacillus* sp and TDG3 showed 100% similarity with *Aeromonas hydrophila*.

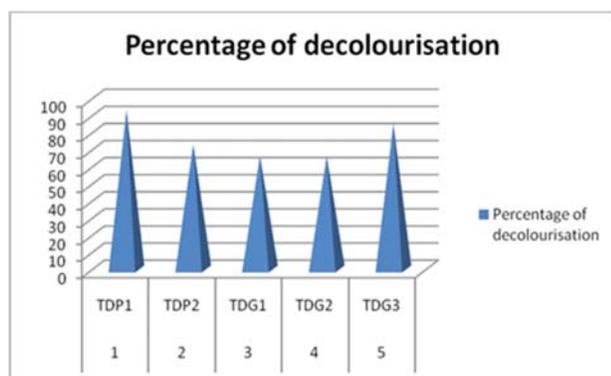
### Decolourization of dye

The decolourisation time showed a relationship with the chemical structure of dyes. The dye Provisional pink was decolourized within 5 days. The percentage of decolourisation was 93% for strain TDP1, the strain TDP2 showed 73% of decolourisation. The percentage of decolourisation of dye Green PB was 85% for the strain TDG3, the strain TDG1 showed 66.4% of decolourisation and the strain TDG2 showed 66% of decolourisation (Table 1) (Figure 1). Complete decolorization of provisional pink by TDP1 was observed after 5 days of inoculation (figure 2).

Complete decolorization of Green PB by TDG3 was observed after 7 days of inoculation (figure 3).

**Table 1:** Decolourisation of Dye

Time (hours)	OD at 592nm				
	TDP1	TDP2	TDG1	TDG2	TDG3
	Provisional pink (50mg/l)		Green PB (50mg/l)		
24	2.713	2.856	2.934	2.920	2.892
48	2.012	2.457	2.670	2.509	2.501
72	1.512	1.892	1.976	1.876	1.765
96	0.914	1.023	1.502	1.490	1.045
120	0.211	0.806	1.012	1.006	0.450
Percentage of decolourisation	93	73	66.4	66	85



**Fig 1:** Percentage of decolourisation by dye degrading strains



**Fig 2:** Decolourisation of textile dye Provisional pink (50mg/l) by TDP1 (*Bacillus sp*) after 5 days of inoculation



**Fig 3:** Decolourisation of textile dye Green PB (50mg/l) by TDG3 (*Bacillus sp*) after 5 days of inoculation

### Decolourization at different dye concentration

Percent decolorization of Provisional pink by TDP1 was varied with initial dye concentrations (50–250 mg l<sup>-1</sup>) when studied up to one week under static condition. At 50 mg l<sup>-1</sup> dye concentration, 93% dye removal was observed; 59% removal was observed at the concentration of 100mg, 40% at 150mg concentration, 33% removal at 200mg concentration and 24% removal at 250mg concentration was recorded with 30 ml batch culture in nutrient broth. Decolorization of Provisional pink by TDP2 was varied with dye concentrations (50–250 mg l<sup>-1</sup>). At 50 mg l<sup>-1</sup> dye concentration, 70% dye removal was observed; 55% removal was observed at the concentration of 100mg, 40% at 150mg concentration, 12% removal at 200mg concentration and 9% removal at 250mg concentration was recorded with 30 ml batch culture in nutrient broth.

Percent decolorization of Green PB by TDG1 was varied with initial dye concentrations (50–250 mg l<sup>-1</sup>) when studied up to one week under static condition. At 50 mg l<sup>-1</sup> dye concentration, 75% dye removal was observed; 52% removal was observed at the concentration of 100mg, 42% at 150mg concentration, 11% removal at 200mg concentration and 9% removal at 250mg concentration was recorded with 30 ml batch culture in nutrient broth. Decolorization of Green PB by TDG2 was varied with dye concentrations (50–250 mg l<sup>-1</sup>). At 50 mg l<sup>-1</sup> dye concentration, 88% dye removal was observed; 56% removal was observed at the concentration of 100mg, 38% at 150mg concentration, 14% removal at 200mg concentration and 10% removal at 250mg concentration was recorded with 30 ml batch culture in nutrient broth. Decolorization of Green PB by TDG3 was varied with dye concentrations (50–250 mg l<sup>-1</sup>). At 50 mg l<sup>-1</sup> dye concentration, 90% dye removal was observed; 62% removal was observed at the concentration of 100mg, 53% at 150mg concentration, 33% removal at 200mg concentration and 30% removal at 250mg concentration was recorded with 30 ml batch culture in nutrient broth.

The decolorization was not prolonged at 250 mg/l, instead this and higher concentration seemed to be toxic for cell growth in 100 ml batch culture.

### Discussion

The textile industry plays an important role in the world economy as well as in our daily life, but at the same time, it consumes large quantities of water and generate huge amount of waste waters. Textile industries consume substantial volumes of water and chemicals for wet processing of textiles. These chemicals are used for desizing, scouring, bleaching, dyeing, printing and finishing. They range from inorganic compounds and elements to polymers and organic products.

### Isolation, identification of dye degrading bacterial strains and decolorization studies of bacterial isolates

Zimmerman *et al.* (1982) isolated the dye degrading six bacterial isolates from sludge samples obtained from various sources including the lake-mud in Tsing Hua University (Hsinchu, Taiwan) and the sludge of wastewater treatment plant in Chang Chun Petro-chemical Co. (Miaoli, Taiwan). White and pink colour colonies surrounded by an almost decolorized zone were isolated and then tested for color removal capability using submerged cultures. Among these colonies, six of them with the highest decolorization ability in SM medium. Decolorization of various dyes was carried

out by growing cells of these six isolates. Among the 24 dyes, Acid Orange 7, Acid Red 106, Direct Orange 39, Direct Yellow 4, Direct Yellow 12, Reactive Black NR, Reactive Blue 160 and Reactive Red 198 were reduced completely by all the strains. Reactive Blue 198 (5- 22%) and Acid Black 172 (0-51%) were reduced only slightly even after 7 days of incubation. The effectiveness of all the six isolates in decolorizing these 24 dyes may depend on the structure and complexity of the dyes, particularly on the nature and position of substituent in the aromatic rings and the resulting interactions with the azo bond.

In the present study soil samples were collected from textile dyeing handlooms and five strains were isolated and designated as TDP1, TDP2, TDG1, TDG2 and TDG3 and they were studied for dye decolorization assay. Among the five strains TDP1 showed maximum decolorization ability.

Sani and Banerjee (1999) found out the decolorization rate of the six isolates DEC1, /DEC4,/DEC2X,/DEC3, DEC5 and/ DEC6 after 1 day of incubation under the same initial cell concentrations. Several biochemical and physiological investigations were conducted to identify the best strain, DEC1. The strain was identified as *Aeromonas hydrophila* according to the GN microplate (Biolog, CA, USA), API 20E (BioMerieux SA, Marcy l'e'toile, France), API 50 CHE (BioMerieux) and partial sequencing of 16S rRNA gene. From phylogenetic analysis based on 16S rRNA sequence, strain DEC1 was also identified as a strain that is most related to *A. hydrophila*.

In the current study, decolorization rate of the five isolates, TDP1, TDP2, TDG1, TDG2 and TDG3 after 5 day incubation under the same initial concentrations was studied. Morphological and biochemical characterization were performed to identify the effective dye degrading strains. Using 16S rRNA sequencing, strain TDP1 was identified as *Bacillus* and strain TDG3 was identified as *Aeromonas sp.*

Knapp *et al.* (1995) investigated the decolorization of molasses wastewater has also been investigated and several fungal cultures capable of decolorization have been isolated. One culture possessing this capability was found to belong to the *Basidiomycete* group. Further screening, utilizing melanoidin, the major coloring substance in molasses, resulted in the isolation of *Coriolus sp.* Its decolorizing ability was associated with the enzyme sorbose oxidase. Subsequent investigation of Ohmomo *et al.*, (1985) resulted in the isolation of *Coriolus versicolor* Ps4a, *Mycelia sterilia* D90; *Aspergillus fumigates* G-2-6 and *A. oryzae*. Several other wood-rotting fungi capable of decolorizing a wide range of structurally different dyes were also isolated and found to be more effective than *P. chrysosporium*.

Raghukumar *et al.* (1996) isolated facultative anaerobic fungi capable of growth on dyes as sole carbon sources have been reported. They, however, do not seem to be able to carry out decolorization. They appear to cleave some of the bonds in these dyes to use carbon sources, yet do not affect the chromophore centre of the dyes. This capability might be of significance when a consortium of microorganisms is employed in degrading dye-containing effluents when other decolorizers are present. Both types, the degrading and the decolorizing microorganisms, would ultimately benefit from each other's activities to achieve complete or faster biodegradation. Success in the decolorization of paper-mill bleach-plant effluent has also been recently reported using unidentified marine fungi which produced the enzymes laccase, manganese peroxidase and lignin peroxidase.

Nigam *et al.* (1996a) Two mixed bacterial cultures, namely PDW and PDC, capable of decolorizing textile dyes were isolated from enrichment cultures that were kept growing in minimal media containing dyes as sole carbon sources and anaerobic conditions for over a year. An investigation into the efficiency of growth and decolorization for these cultures, PDW and PDC, concluded they were facultative, with an ability to grow under both aerobic and anaerobic conditions, but with highest growth rate and decolorization ability under anaerobic conditions. Both growth and decolorization in the two mixed cultures were enhanced in rich media supplemented with yeast extract. A distinct advantage was noticed for culture PDW which, upon purification, was found to be composed of at least two bacterial strains, *Alcaligenes faecalis* and *Comamonas acidovorans*.

Nigam & Marchant, 1995, noted that the two components obtained in pure culture upon the purification of PDW did not have the independent capability to decolorize any of the dyes. The advantages of mixed cultures are apparent as some strains can collectively carry out complex biodegradation tasks than individual strain can achieve independently. Similar other mixed bacterial cultures have also been reported recently (Knapp & Newby, 1995) further testing involved a selection of cheap support media for biofilm development suitable for active textile dye decolorization using the PDW-mixed-bacterial culture was pursued. Several support materials were investigated, including gravel, calcium alginate beads, polystyrene chips, polyurethane foam chips, nylon-web cubes, inert polyethylene chips, sea-shell powder and highly porous volcanic rocks composed of silicon and aluminium oxides.

#### **Decolourization studies of bacterial isolates in different dye concentrations**

Amar Telke *et al.* (2008) *Rhizobium radibacter* efficiently decolorized the increasing concentration of dyes (100, 200, 300, 400, 500 mg/l) with a decolorizing efficiency varying from 60-90%, where the diazo dye Reactive Black 5 decolorization by *Candida oleophila* was significantly inhibited at 300 to 500 mg/l. The time required for decolorization was proportional to dye concentration. The increase in dye has no adverse effect on the growth of an organism. In the present study, all the isolated strains (TDP1, TDP2, TDG1, TDG2 and TDG3) showed decolourization of dyes with varying concentrations (50mg/l – 250 mg/l). Among the five isolated strains, the strain TDP1 showed maximum decolourisation.

#### **Conclusion**

The textile finishing generates large amount of dyes, pigments, dispersing agents salts, leveling agents and heavy metals. Effluent discharge from textile and dye stuff industries to neighbouring water bodies and waste water systems facing health concerns. Most of the dyes are carcinogenic in nature. The ability of the microbes to carry out dye decolourisation has received much attention. In the present study the isolated dye degrading bacterial population from natural environment which can destroy the recalcitrant nature of the dye and made them easily biodegradable.

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