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Prevalence of Bacterial Isolates in Textile Dye Effluent and Analysis of its Dye Degrading Efficiency

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Abstract: Bacterial decolorization is efficient and fast, but individual bacterial strains usually cannot degrade azo dyes completely and the intermediate products are often carcinogenic aromatic amines, which need to be further decomposed. Thus, treatment systems composed of mixed microbial populations achieve a higher degree of biodegradation and mineralization due to the synergistic metabolic activities of the microbial community and have considerable advantages over the use of pure cultures in the degradation of synthetic azo dyes. In the present study, dye house effluent was collected from a dyeing unit in Theco Silks, Thirubhuvanam region, Kumbakonam district, Tamil Nadu, India. It was refrigerated at 4°C and used without any preliminary treatment. Six different bacterial isolates *viz*., *Bacillus odyssey, Bacillus thuringiensis, Bacillus subtilis, Bacillus cereus, Alcaligenes* sp. and *Nocardiopsis alba* were isolated from the textile dye effluent sample. The bacterial isolates were isolated by Serial dilution technique (Pour plate method). The bacterial isolates were identified in genus level by the Colony morphology, Preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Catalase and Oxidase, Plating on selective medias and by performing biochemical tests. The identified bacterial isolates were screened for its decolourization of Reactive dyes by Plate assay. The bacteria *Nocardiopsis alba* showed maximum zone formation when compared to other bacterial isolates.

Key words: Bacteria · Textile Dye · Degradation and Dye Effluent

INTRODUCTION

Environmental biotechnology is constantly expanding its efforts in the biological treatment of dye-contaminated wastewaters. Bacteria and fungi were widely used for decolorization of textile dyes [1]. The ubiquitous nature of bacteria makes them invaluable tool for bio treatment. Although, numerous bacterial species can decolorize textile dyes, only a few are able to mineralize these compounds into CO_2 and H_2O. Because, the effectiveness of microbial decolorization has depends on the adaptability and the activity of selected microorganisms. Therefore, exploring the bacterial community from dye enriched environment and its further characterization is a crucial step to discover a novel biocatalyst. The identification of potential dye-decolorizing bacterial species requires a screening method.

Industrial wastes and effluents are undesirable byproducts of economic development and technological advancement. When improperly handled and disposed, industrial wastes imperil both human health and the environment. Human exposures (occupational and non- occupational) to industrial wastes have led to health effects ranging from headaches, nausea, lung and skin irritations, to serious ailments like congenital malformations [2- 5].

Water pollution control is at present one of the major areas of scientific activity. While coloured organic compounds generally impart only a minor fraction of the organic load to wastewater, their colour renders them aesthetically unacceptable. Colour is one of the most obvious indicators of water pollution and discharge of highly coloured synthetic dye effluents can be damaging to the receiving water bodies [6]. Textile dyes are one of the most prevalent type chemicals in use today.

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Around 10,000 different dyes with an annual production of more than $7 \cdot 10^5$ metric tonnes worldwide are commercially available [7].

Dyes are an important class of chemicals which are widely used in many industrial processes, like in leather, textile and printing, food and cosmetics industries. Most of these dyes are synthetic in nature and are classified based on their chemical structures into 6 different classes as azo, anthraquinone, sulfur, indigoid, triphenylmethane and phthalocyanine derivatives. Due to the extensive use of these dyes in industries, they become an integral part of industrial wastewater. In fact, of the 4,50,000 tons of organic dyes annually produced worldwide, more than 11% is lost in effluents during manufacture and application processes [8]. The present study was focused on prevalence of bacterial isolates in textile dye effluent and analysis of its dye degrading efficiency.

MATERIALS AND METHODS

Collection of Textile Dye Effluent: The dye house effluent was collected from a dyeing unit in Theco Silks, Thirubhuvanam region, Kumbakonam district, Tamil Nadu, India. It was refrigerated at 4°C and used without any preliminary treatment.

Dyes Used: Reactive azo dyes were used in this study. The dye samples were commercially graded and supplied by the dealers of "SIGMA Aldrich, U.S.A". Reactive azo dyes used in this research were,

- Reactive Orange-16
- Reactive Black-B
- Reactive Yellow-MR
- Reactive Blue-MR
- Reactive Red-M5B

Isolation of Bacterial Isolates from Textile Dye Effluent: The bacterial isolates present in the textile dye effluent were isolated by Serial dilution (Pour plate) technique. 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 concentrations of samples were transferred to sterile petriplates and evenly 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.

Maintenance of Bacterial Isolates: Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored at 4°C.

Identification of Bacteria Isolated from Textile Dye Effluent: Identification of the bacterial isolates was carried out by the routine bacteriological methods *i.e.*,

- By the colony morphology
- Preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Catalase and Oxidase.
- Plating on selective medias.
- By performing biochemical tests.

Screening of Bacterial Isolates for the Decolourization of Reactive Dyes by Plate Assay: The decolourization of textile Reactive azo dyes by bacterial isolates was determined by Plate assay technique. The Plate assay was performed for the detection of decolorizing activity of bacteria isolated and identified from the textile dye effluent. The Nutrient agar and Reactive dyes (500 mg/l) was autoclaved at 121°C for 15 min. The bacterial cultures were platted on Nutrient agar plates containing Reactive azo dyes. The plates were wrapped with parafilm and were incubated in incubator at 37°C for 4 days. The plates were observed for clearance of the dye surrounding the colonies.

RESULTS

Identification and Characterization of Bacteria Isolated from Textile Dye Effluent: Six different bacterial isolates were isolated and identified from the textile dye effluent. The characteristics of the identified bacterial isolates were furnished in Table-1. The isolated bacterial isolates were identified and characterized as *Bacillus odyssey*, *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus cereus*, *Alcaligenes* sp. and *Nocardiopsis alba*. All the bacterial isolates except *Alcaligenes* sp. showed Gram positive reaction.

Table 1: Identification and characterization of bacteria isolated from textile dye effluent

	Biochemical and physiological characterization of selected strains					
Character	*Bacillus cereus*	*Bacillus odyssey*	*Bacillus subtilis*	*Bacillus thuringiensis*	*Alcaligenes* sp.	*Nocardiopsis alba*
Gram staining	Gram positive rod	Gram positive rods.	Gram positive rods.	Gram Positive rods	Gram-negative rods.	Gram Positive
Endospore	Central spores present	Round terminal endospore.	Central spores present	Terminal endospore	No spores	No spores
Motility	Positive	Motile	Non-motile	Positive	Positive	Non motile
Catalase	Positive	Positive	Positive	Positive	Positive	Positive
Oxidase	Positive	Positive	Negative	Negative	Positive	Positive
Nutrient agar	Dull or frosted appearance	Round, smooth, flat with entire edges and beige in color.	Colonies are large, circular or irregular, grey-yellow, granular and difficult to emulsify.	Colonies are smooth, circular, white-cream, entire, opaque.	Colonies are circular non pigmented to grayish white, translucent or opaque, flat to convex, margin is entire	Colonies are dirty white aerial mycelium becoming light-yellowish grey in ageing cultures.
MacConkey agar	No growth	Non-lactose fermenting colonies	Non-lactose fermenting colonies	No growth	Non-lactose fermenting colonies	Non-lactose fermenting colonies
Glucose fermentation	Acid produced.	Negative	Acid produced	Acid produced	Negative	Acid produced
Mannitol fermentation	Negative	Negative	Acid produced	Negative	Negative	Acid produced
Sucrose fermentation	Negative	Negative	Acid produced	Negative	Acid produced	Acid produced
Xylose fermentation	Negative	Negative	Acid produced	Negative	Acid produced	Acid produced
Indole	Negative	Negative	Negative	Negative	Negative	Negative
Methyl Red Test	Positive	Negative	Negative	Positive	Positive	Negative
Voges Proskauer Test	Negative	Negative	Positive	Positive	Negative	Negative
Citrate utilization	Negative	Negative	Positive	Negative	Negative	Negative
Nitrate reduction	Negative	Negative	Positive	Positive	Negative	Positive
Gelatin hydrolysis	Positive	Negative	Positive	Negative	Negative	Positive
Starch hydrolysis	Positive	Negative	Positive	Negative	Positive	Positive
Urease	Negative	Negative	Negative	Positive	Negative	Negative

Table 2: Screening of bacterial isolates for dye degradation by plate assay

		Zone formation (in mm)				
S.No	Bacterial Isolates	Reactive Orange-16	Reactive Black-B	Reactive Yellow-MR	Reactive Blue-MR	Reactive Red –M5B
1	*Bacillus odyssey*	35	34	33	31	29
2	*Bacillus thuringiensis*	33	31	30	28	24
3	*Bacillus subtilis*	30	29	27	25	20
4	*Bacillus cereus*	28	26	24	20	16
5	*Alcaligenes* sp.	27	24	21	17	13
6	*Nocardiopsis alba*	25	21	19	13	8

Screening of Bacterial Isolates for the Decolourization of Reactive Dyes by Plate Assay: The bacterial isolates were screened for the decolourization of reactive dyes by Plate assay and the results were tabulated in Table-2. The identified bacterial isolates *viz*., *Bacillus odyssey*, *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus cereus*, *Alcaligenes* sp. and *Nocardiopsis alba* were used for Plate decolourization assay. Maximum decolourization was recorded by *Bacillus odyssey* in the plate containing Reactive Orange-16 (35 mm) followed by *Bacillus thuringiensis* (33 mm), *Bacillus subtilis* (30 mm), *Bacillus cereus* (28 mm), *Alcaligenes* sp. (27 mm) and *Nocardiopsis alba* (25 mm). The zone of inhibition in the plates containing the remaining reactive dyes was also recorded by the bacterial isolates in the above given order. Next to Reactive Orange-16, the bacterial isolates showed maximum zone of inhibition in the plate containing Reactive Black-B followed by Reactive Yellow-MR, Reactive Blue-MR and Reactive Red M5B.

DISCUSSION

In the present study, six different bacterial isolates were isolated and identified from the textile dye effluent. The isolated bacterial isolates were identified and characterized as *Bacillus odyssey*, *Bacillus thuringiensis*, *Bacillus subtilis*, *Bacillus cereus*, *Alcaligenes* sp. and *Nocardiopsis alba*. All the bacterial isolates except *Alcaligenes* sp. showed Gram positive reaction. Saranraj *et al*. [10] reported that six isolates from different sources including lake-mud and wastewater treatment plant sludge showed various decolorization efficiencies for di-azo dyes.

Abd El-Rahim and Moawad [11] and Saranraj *et al*. [12] have reported isolation of organisms adapted to high dye concentration from sites near textile industries complex. The selected isolate is a sporulating Gram positive motile rod, occurring singly, grew as rough colony on nutrient agar. On the basis of conventional

biochemical tests, it was identified as *Bacillus cereus* or *Bacillus thuringiensis*. Staining of the parasporal body showed its presence, which indicated the identity of the isolate as *Bacillus thuringiensis.*

Tan *et al*. [13] studied in detail the dynamics of microbial community for X-3B wastewater decolorization under high salt and metal ions conditions. Khalid *et al*. [14] reported decolorization of azo dyes under high salt concentrations by *Shewanella* sp. The application of microorganisms for the biodegradation of synthetic dyes is an attractive and simple method by operation. However, the biological mechanisms can be complex. Large number of species has been tested for decoloration and mineralization of various dyes. Unfortunately, the majority of these compounds are chemically stable and resistant to microbiological attack. The isolation of new strains or the adaptation of existing ones to the decomposition of dyes will probably increase the efficacy of bioremediation of dyes in the near future.

The bacterial isolates were screened for the decolourization of reactive dyes by Plate assay. Maximum decolourization was recorded by *Bacillus odyssey* in the plate containing Reactive Orange-16 (35 mm) followed by *Bacillus thuringiensis* (33 mm), *Bacillus subtilis* (30 mm), *Bacillus cereus* (28 mm), *Alcaligenes* sp. (27 mm) and *Nocardiopsis alba* (25 mm). The zone of inhibition in the plates containing the remaining reactive dyes was also recorded by the bacterial isolates in the above given order. Next to Reactive Orange-16, the bacterial isolates showed maximum zone of inhibition in the plate containing Reactive Black-B followed by Reactive Yellow-MR, Reactive Blue-MR and Reactive Red M5B.

Burchmore and Wilkinson [15] studied the zone of inhibition with control dyes (Crystal violet, Phenol red, Malachite green, Methyl green and Fuchsin) with *Staphylococcus epidermidis* strains at a concentration of 100 ppm and at a concentration of 500 ppm. Whereas, degradation products did not show growth inhibition. These findings suggest the non-toxic nature of the product formed. Previous reports showed Malachite green and Crystal violet degradations into leuco-malachite and leuco-crystal violet are equally toxic to Malachite green and Crystal violet [16, 17].

CONCLUSION

From this present study, it was concluded that the bacterial isolates like *Bacillus odyssey, Bacillus thuringiensis, Bacillus subtilis, Bacillus cereus, Alcaligenes* sp. and *Nocardiopsis alba* were predominantly present in textile dye effluent and they were used as a good microbial source for the textile Reactive dye decolourization and waste water treatment in textile dye industries. The bacteria *Nocardiopsis alba* showed maximum zone against textile Reactive dyes followed by *Bacillus subtilis, Bacillus cereus, Alcaligenes* sp., *Bacillus odyssey* and *Bacillus thuringiensis*. Among the five dyes tested, the dye Reactive Orange-16 showed maximum zone when compared to other reactive dyes.

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