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Research Article

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Bio-degradation of harmful textile dyes by marine bacteria from Tuticorin coastal Waters Southeastern India

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ABSTRACT

The minimum and maximum level of dye degrading bacterial density was found to be 2.1 X 103 CFU/g and 8.5 X 103 CFU/ respectively. The strains were tested for their degradation efficiency using 6 dyes namely Malachite green, Majenta MB, Turquoise blue H, Orange Fast Boardeux r GB, Direct blue 2B and Direct Congo Red. Among the 5 strains, MSB 4 exhibited maximum degradations activity against all the six dyes (Malachite green – 6 mm, Majenta MB, Turquoise blue Ea, Orange fast boardeux – 4 mm and Direct blue 2B, Direct Congo Red – 2mm). The active strain MSB4 was used for qualitative estimation of degradation of dyes at different concentrations. The percentage of dye discoloration of dye was observed against Majanta MB (68.25%) followed by orange Fast boardeux (64.10%), Congo red (63.55%), Malachite gren (61.89%) and Direct blue (22.09%), while lowest discoloration was observed against Turquoise blue (12.06%) at 100ppm concentration. At 500 ppm concentration the maximum percentage of dye discoloration was observed against direct blue (9.03%). The active strain MSB4 was further identified as Pseudomonas sp based on the morphological and biochemical tests. This work concludes that the Pseudomonas sp isolated from mangrove sediment have the ability to degrade harmful dyes such as Direct blue, Direct Congo Red, Turquoise blue H, Orange fast boardeux GB and Malachite green which could be considered for the bio-degradation of colour effluent discharged from paper and printing industries. Further works has to be done to optimize the discoloration effect this bacterium.

Key words: Marine bacteria, bio-degradation, textile dyes

INTRODUCTION

The treatment of such dye containing effluent was initially carried by using physical and chemical treatment processes including flocculation, flotation, electro flotation, membrane-filtration, ion exchange, irradiation, precipitation, ozonation and adsorption using activated carbon [1-2]. But with time, potential hazards and disadvantages of these methods were noted as, formation of toxic sludge and formation of even more toxic metabolites. Biological treatment methods are more desirable as they are environmentally friendly, do not produce secondary pollutants and have a higher possibility of wider application [3]. However, viable biological treatment using microorganisms requires cheap carbon sources.

The marine environment provides unique and specific composition of both organic and inorganic substances for expansion of microorganisms [4]. The resident of marine microorganisms in seawater, unlike air, typically contains 107 viruses, 106 bacteria, 103 fungi and microalgae/ml. The ecological niches such as deep sea, hydrothermal vents, mangrove forests, algae, sponge and fish provide habitats for the evaluation of specific microorganisms. In Tuticorin coast, the mangrove ecosystem is considered as a crucial one in ecological perspective [5-6]. The microbial diversity is vast in mangrove environment of this area; however studies related to degradation of hazardous wastes by micro organisms are scanty. Hence this study has been aimed to isolate the degrading bacterial strains from mangrove associated sediment collected from nearby Roche Park, coastal area of Tuticorin. Further to assess the efficiency of selected bacterial strain in degradation of dyes in different concentration.

EXPERIMENTAL SECTION

Sample collection

The sediment sample was collected from nearby Roach Park area (N 08˚46' 582 and E 78˚ 09'363) of Tuticorin coast, southeast coast of India. Sediment sample was collected at 1m depth from surface using the sterile polyvinyl corer (10cm dia) and transferred to sterile vials and tightly sealed. The collected sample was brought to the lab in an ice-box.

Isolation of dye-degrading bacteria

The isolation of bacteria was performed by the dilution plate method. 1 gm of sediment sample was serially diluted and 0.1 ml of aliquots was plated separately on Zobell Marine Agar media incorporated with various dyes such as Malachite green (Oxalate), Majenta MB, Turquoise blue H, Fast boardeux GB, Direct blue 2B and Direct Congo red at 100 ppm concentration. All the dyes were procured from commercial dye trader, Rajapalayam, Tamilnadu. Plates were maintained in triplicate.

The plates were incubated at room temperature for 48 hrs. The colonies were counted and expressed as Colony Forming Units (CFU) per gram. Based on the different morphology and appearance of distinct mild zone around the colonies, 5 strains were selected and purified by streaking method and the pure isolates were kept on slants at 40 C for further studies. The isolated bacterial strains were given designated codes.

Degradation activity of marine bacteria

The isolated 5 strains were inoculated into 100 ml ZMB broth, and incubated in a shaker at 120 rpm for 48 hrs. The decolorization of dyes was carried out using well diffusion method. 100 µl of broth culture of each strain was loaded into respective 5 mm well of the medium incorporated with all the six dyes at 100 ppm concentration. The zone of degradation was measured from the edge of the well to end of the clear zone in millimeter.

Estimation of dye discoloration

The 100 ml of Zobell marine broth was incorporated with all the six dyes at the concentration of 100 and 500 ppm separately. 24 hour old bacterial culture of MSB4 strain was inoculated into the dye incorporated culture broths at 5 % (v/v) separately. All the broth cultures were incubated on a shaker at 120 rpm for 48 hrs. At the end of incubation period the broth was centrifuged at 10000 rpm for 15 minutes to separate the pellet and supernatant. The supernatant was analyzed spectrophotometrically. The maximum absorbance of each dye incorporated broth without bacterial culture (control) was determined which was used to analyze the supernatant.

$$\text{Percentage of discoloration} = \frac{\text{Initial absorbance value} - \text{Final absorbance value} \times 100}{\text{Initial absorbance value}}$$

Identification of dye degrading bacterium

The active bacterial strain (MSB4) was identified up to genus level based on the morphological and biochemical characteristic features following the procedures given in Bergey's manual.

Antibiotic sensitive test

Disc diffusion or the Kirby-Bauer test is mostly used for the antibiotic sensitive test. Each isolated culture was inoculated in 5 ml marine broth and incubated at 370C for 24 hrs. Sterile cotton swabs were swabbed with the culture, gently pressed against the wall of the test tubes to remove excess bacterial inoculums and swabbed on the air-dried Zobell marine agar plates aseptically. Using flamed forceps the commercial penicillin antibiotic discs were

transferred to the agar plates. After overnight incubation at 370C, the antibiotic sensitivity of culture was determined by measuring the zone of inhibition.

RESULTS

The total dye degrading bacterial density enumerated from mangrove sediment is given in table 1. The minimum and maximum level of dye degrading bacterial density was found between 2.1 X 103 CFU/g and 8.5 X 103 CFU/ respectively. Based on the formation of mild zone around the colonies, five dye degrading bacterial strains were randomly selected and screened for their efficiency in degrading dyes (Tab.1)

Table 1: Total dye resistant bacterial density

Isolation Media with dye	Dye degrading bacterial density (CFU/g)
Malachite green+ Zobell Marine Agar	3.5x103
Majenta MB + Zobell Marine Agar	5.4x103
Turquoise blue H + Zobell Marine Agar	2.1x103
Orange fast boardeux GB + Zobell Marine Agar	4.9x103
Direct blue 2B + Zobell Marine Agar	8.1x103
Direct Congo red + Zobell Marine Agar	8.5x103

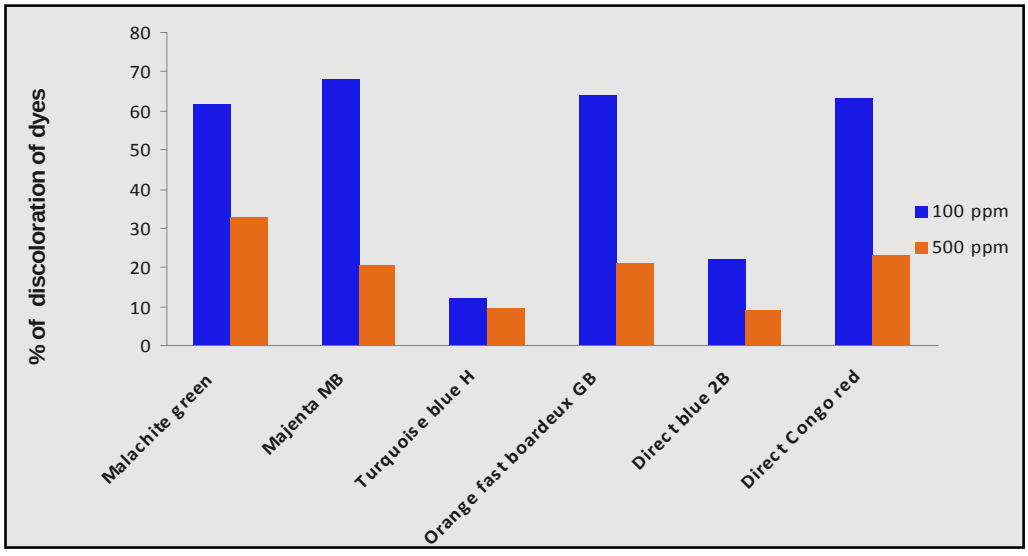
Dye degrading activity

In the preliminary screening, invariably all the five strains showed degradation activity. Among them, the strain MSB4 showed (Table 2 & Fig.1) higher level activity against all the dyes. The zone of inhibition ranged from 2 to 6 mm. The maximum level of degradation was observed against Malachite green (6mm) and minimum against Direct blue and Congo red (2 mm). The MSB1, MSB2 and MSB5 bacterial strains also showed degradation activity but the activity was considerably low when compared with MSB4, while MSB3 bacterial strain exhibited activity against four dyes. Based on the observed degradation zone, active bacterial strain MSB4 was selected for the further qualitative estimation.

Table 2: Dye degradation activity of marine bacteria isolated from mangrove sediment

S. NO	Dyes	MSB1	MSB2	MSB3	MSB4	MSB5
		(Dye degradation zone in mm at 100 ppm concentration)				
1	Malachite green	4	5	3	6	4
2	Majenta MB	2	2	2	4	4
3	Turquoise blue H	2	1	-	4	2
4	Orange fast boardeux GB	2	2	1	4	1
5	Direct blue 2B	2	1	1	2	1
6	Direct Congo red	-	-	-	2	-

Fig. 1: Dye discoloration effect of MSB4 strain



In generally, the percentage of dye discoloration was inversely proportional to the concentration of dyes (Table 3 and Fig.2). The observed result showed that the intensity of degradation of dyes was increased after the marine bacterial treatment. The highest percentage of dye discoloration was observed against Magenta MB (68.25%) followed by orange fast boardeux (64.10%), congo red (63.33%), Malachite green (61.99%) and Direct blue (22.09%) while lowest discoloration was observed against Turguoise blue (12.06%) at 100 ppm concentration. At 500 ppm concentration, the maximum percentage of dye discoloration was observed against malachite green (32.80%) and minimum against direct blue (9.03%).

Table 3: Dye degradation effect of MSB4 strains against at different concentration

S. NO	Dyes	Absorption maxima (λm)	Percentage of discoloration	
			100ppm	500ppm
1	Malachite green	440	61.99	32.80
2	Majenta MB	520	68.25	20.34
3	Turquoise blue H	490	12.06	9.30
4	Fast boardeux GB	495	64.10	21.12
5	Direct blue 2B	589	22.09	9.03
6	Direct Congo red	495	63.33	23.21

Fig.2: Discoloration effect of MSB4 strain

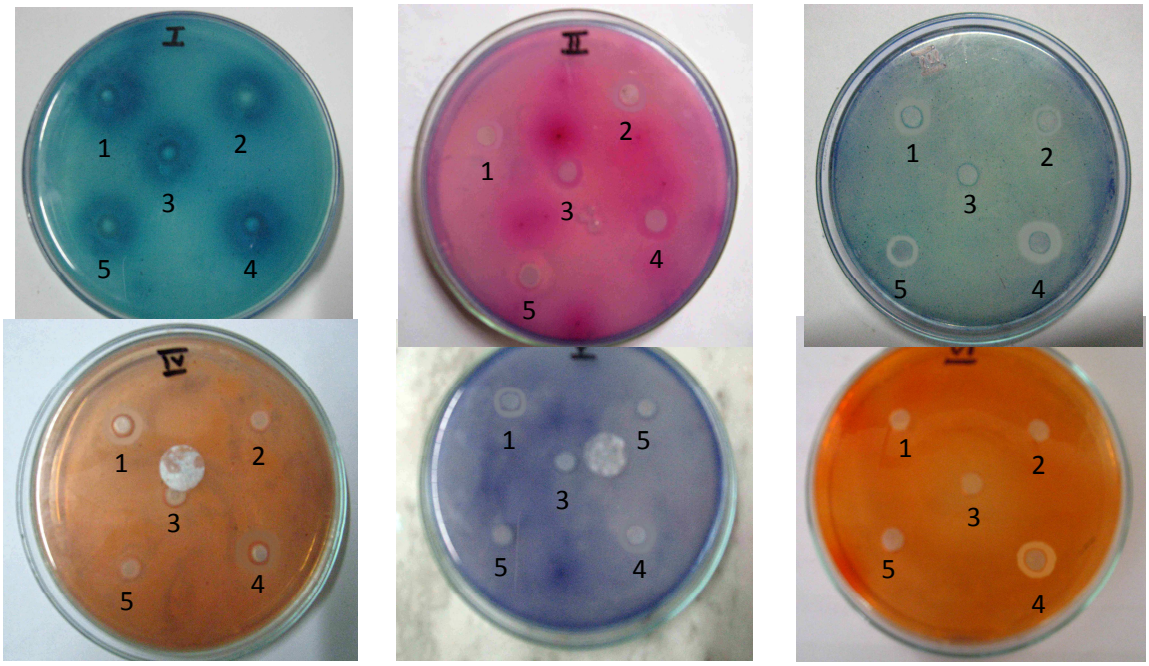


Table 4: Morphological and biochemical feature of active dye degraded bacterium

Morphological and biochemical test	***Pseudomonas sp.*(MSB4)**
Gram staining	Rod/negative
Motility	Motile
Indole	-
Methyl red	-
Voges proskaur	-
Citrate	+
Oxidase	+
Catalase	+
Carbohydrate fermentation of glucose	-
Sensitivity to penicillin disc	-
Lipid hydrolysis	+
Gelatin hydrolysis	+

Identification of dye degrading bacterium
The active dye degraded bacterial strain (MSB4) was identified up to genus level based on the morphological and biochemical characters (Table 4). The strain was identified as *Pseudomonas* sp.

Identification of Pseudomonas sp. (MSB4)
As the strain of *Pseudomonas* sp. showed pink colour for gram staining, this was identified as gram-negative rod. The stain was motile. When carbohydrate fermentation test was performed, they did not ferment the glucose or produced gas and acid. In oxidase test, the strain was positive as change of color was observed. In catalase test, formation of effervescence was observed. It also showed positive result in gelatin and lipid hydrolysis. It showed negative result in indole, MR-VP and positive in citrate test by color change from green to Prussian blue. The stain was resistant to the penicillin antibiotic. But, they grew in cetrimide agar plate and produced blue green pigment.

DISCUSSION

Marine microorganisms have developed unique metabolic and physiological capabilities that not only ensure survival in extreme habitats but also offer the potential for the production of metabolites, which would not be observed, from terrestrial organisms [7-9]. In this study, dye degrading bacterial density varied between 2.1 X 103 CFU/g (blue) to 8.5 X 103 CFU/g (Congo red) in mangrove sediment samples. It suggests that the mangrove sediment associated bacteria may have the ability to degrade or resist the dyes. Some works have been done on isolation of dye degrading bacteria from dye contamination soil. Mariappan *et al*. (2003) [10] have recorded an amount of THBP in the azo dye contaminated soil ranging from 13.2 X 107 (site 4) to 32 X 107 (site 1). In these works, the occurrence of high bacterial load in the dye-contaminated soil was reported to be higher than this present study; this may be either due to the enrichment of the dye degradable population or the dilution of the dye which might have lowered the toxicity of the dye.

The present study, six dyes were subjected to evaluate the dye degradation activity of five individual strains which all were isolated from mangrove ecosystem. Among them, one strain (MSB4) showed prominent degradation against all the dyes. The zone of inhibition was ranging from 2 to 6 mm. The maximum level of degradation was observed against Malachite green (6 mm) and minimum against direct blue and Congo red (2 mm). The present observation was corroborated with Gayathri et al. (2010) [11] who have reported that the endophytic bacteria isolated from mangrove plant showed good degradation effect against malachite green. This observed discoloration effect could be attributed to the production of some degradative enzymes by marine bacteria. The percentage of dye discoloration was inversely proportional to the concentration of dye. Similar observation was made during discoloration of malachite green by *Kocuria rosea* MTCC 1532 strain [12].

The potent dye degrading bacterial strain (MBS4) was identified as *Pseudomonas* sp. based on the colony morphology and biochemical characteristics [13]. A wide range of bacteria were studied for their ability to decolourise synthetic dyes. Some of these strains included *Aeromonas hydrophila* [14], *Pseudomonas luteola* [15], *Escherichia coli* NO3 [16] and *Pseudomonas mendocina* MCM B-402 [17]. The highest dye degrading effect was observed in *Pseudomonas* sp. it suggests that which can be regarded as an important candidate from the group of marine bacteria, applicable to efficient removal of synthetic dye from effluents. Further, excessive use of synthetic chemical dyes should be restricted. They should be replaced by vegetable dyes, which are eco-friendly.

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