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Dye-decolorization by native bacterial isolates, isolated from sludge of carpet industries Bhadohi- India

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ABSTRACT

In the present study, sludge samples were collected from the place where effluents were released by the carpet industries in Bhadohi district. Physico-chemical analysis revealed sludge samples were dark brown in color, pH (6.91 to 9.5), ECe (5.69-11.50 dSm^{-1}), organic C (4.40 - 12.60%), N content (3.50 -13.90%), PO_4 content (7.25-13.34%) and K_2O content (1.02 -3.21%). Maximum population of bacterial (cfu≈10^6/g) isolates in sludge samples were found on NA media followed by Kings' B and NMS. Out of 86 bacterial isolates, 39, 28 and 19 isolates were isolated on NA, Kings' B and NMS agar media respectively. Sixteen bacterial isolates decolorized the test dyes in respective media plates, 10 decolorized congo red (CR) and 6 decolorized trypan blue (TB) up to higher concentration (25 to 150 ppm) of dyes in agar plate. *Pseudomonas* sp., *Bacillus* sp. and *Azatobacter* sp. and their consortium showed significant decororization of both the dyes after 24h in Nutrient broth. Consortium of C3 (*Bacillus* sp. + *Azatobacter* sp.) decolorized 94% of 100 ppm CR and 95% of TB.

1) INTRODUCTION

Carpet and textile industrial effluent accounts for the largest proportion of dyes pollution worldwide, in the process of fabrics coloration are not efficient to use total dyes concentration and lost 10 to 40% of the dyes in the effluent [1]. Disposal of these dyes into the environment causes serious damage, since they may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration [2] and also they may be toxic to some aquatic organisms due to their breakdown products [3]. The three most common groups are azo, anthraquinone and phthalocyanine dyes [4], most of which are toxic and carcinogenic [1]. Biggest carpet manufacturing centers in India is district Bhadohi [25°25′N 82°34′E25.42°N 82.57°E], and dominant practice to make the carpets overhear are hand-knotted carpet. Bhadohi region is the largest handmade carpet weaving cluster, engaging more people in the macro and micro industries. Most of the carpet manufacturers directly flush on their effluent on the environment without any proper scientific treatment. Azo dyes are considered as electron-deficient xenobiotic compounds because they possess the azo (N=N) and sulfonic ($-SO_3^-$) electron-withdrawing groups, generating electron deficiency in the molecule and making the compound less susceptible to oxidative catabolism by bacteria. As a consequence, azo dyes tend to persist under aerobic environmental conditions [5]. Congo red (sodium salt of benzidinediazo-bis-1-naphtylamine-4 sulfonic acid) has been reported to be a carcinogenic direct azo dye used for colouration [6]. Trypan blue is an azo dye derived from toluidine. It is a vital stain used to selectively of colour it considered to be recalcitrant, non biodegradable and persistent [7]. Extensive work has been carried out on the pollution problems associated with the discharge of dye effluent from industries. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Many microorganisms are capable of degrading the dyes, including bacteria [8], fungi [9-10], yeast [11-12], and actinomycetes [13]. This part of the study was undertaken to native bacterial isolate from sludge of carpet industry situated at district Bhadohi (UP) region, capable to decolorize azo dyes, Trypan blue (TB) and Congo-red (CR).

2) MATERIAL AND METHODS

2.1 Sites, sample collection, physico- chemical analysis and bacterial isolation: Fifteen survey sites (**Table 1**) of districts Bhadohi, were selected in the present study. Each site was 4 to 20 Km apart from each other. 100gm of sludge samples were taken in sterile polythene packets and kept at 4°C for further analysis. The physico-chemical characteristics of sludge

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samples like Color, pH, ECe (Electrical conductivity), OC (Organic carbon), N, PO_4 and K_2O were analyzed by the method of APHA [14]. 10g of sludge from each sample were added to 90 ml of sterile saline [0.8% NaCl (w/v)] water and shaken for 30 minutes through mechanical shaker. Ten fold dilutions were made and plated on to three different media viz., NA (Nutrient agar), King's B medium [15] and NMS (Nutrient mineral Salt medium). Morphologically different bacterial isolates were picked from respective media plates after 24h and stored at 4^0C for further examination.

2.2 Screening of dye decolorizing bacterial isolates: The decolorization activity of the bacterial isolates were examined by spot test against the synthetic dyes, each bacterial isolates were grown in the 10 ml test tubes of respective broth media (24h at 38^0C $\pm 2^0$ C, rpm=150) and 2µl of bacterial inoculum was spotted on pre prepared NA medium plates amended with various concentrations (25ppm to 150ppm) of two different test dyes *viz.* TB and CR. Control plates were also maintained without bacterial inoculums at 38^0C $\pm 2^0$ C. Clear zone indicated the dye-decolorization activity and zone was measured by HiAntibiotic Zone Scale^{TM}-C and best zone producing isolates were further inoculated in the test tube of NB (Nutrient broth) supplemented with different concentrations of the test dyes and incubated at 38^0C $\pm 2^0$ C under shaking condition (200rpm). The bacterial cell concentration was determined using OD of the culture at 600 nm (OD_{600}) [8]. Aliquots (3 ml) of the culture media were drawn after regular intervals for color measurement. Suspended particles from the culture medium were removed by centrifugation at 7,000 rpm for 20 min. Decolorization was monitored by earlier described [16, 17] measuring the absorbance of maximum peak wavelength λ=497nm for CR and λ=595nm for TB respectively by using UV-Vis spectrophotometer (model: ELICO-SL218-double beam). A standard graph for absorbance versus dye concentration for each dye was obtained by plotting the corresponding maximum absorbance in the UV–Vis spectra at different dye concentrations prepared by dissolving the dye in distilled water. Un-inoculated culture media with and without added dyes were used as negative controls. The decolorization efficiency of different isolates was expressed as [Decolorization (%)=(A_0-A) / A_0 X 100], where A_0 is the initial absorbance and A is the absorbance of medium after decolorization at the K_{max} (nm) of each dye. Identification of bacterial isolates was done through the methods of Bergey's Manual.

2.3 Statistical analyses: The data obtained were subjected to ANOVAs, and means were compared with Duncan's multiple range test. All statistical analyses were conducted using SPSS (Version 14; IBM, Armonk, NY, USA).

3) RESULTS AND DISCUSSION

The sludge-soil samples were collected from fifteen different sites of effluent drain areas of carpet industries from Bhadohi district. Physico-chemical parameters of sludge were analyzed and the respective data is given in **table 1.** Most of the sludge samples were dark brown (DB) in color with ECe (Electrical conductivity:dSm^{-1}) ranged 5.69-11.50, pH ranging from 6.91 to 9.5, and organic C from 4.20 to 12.60%, percent N content 3.50 -13.90, percent PO_4 content 7.25-13.34 and percent K_2O content 1.02 -3.21. Several workers reported effluent drain from carpet industries have good amount of dye along with other elements [1]. Rich sources of organic carbon, nitrogen, phosphorous and potassium in sludge samples were able to facilitate the growth of microorganism [3]. A total of 86 bacteria were isolated from the sludge from all the fifteen of district Bhadohi on three different media. The populations of bacteria from each sludge sample on different media are presented in **table 1**.

On the basis of morphological and microscopic examination, thirty nine, twenty eight and nineteen isolates were isolated on NA, Kings' B and NMS agar media respectively. Bacterial population (g^{-1} sludge sample) were examined and represented in Cfu (Colony forming Unit), maximum population of bacteria were found to be with NA media (30-100 x 10^4) followed by KB (27-62 x 10^4) and NMS (12-78 x 10^4) (**Table 1**). Screenings of different bacterial isolates on different media were earlier reported [18]. The decolorizing ability of native bacterial isolates was screened for two synthetic azo dyes (TB and CR) through spot test; similar approach was applied to screen the bacterial isolates by Upadhyay et al. [18] for salinity tolerant. Out of 86 bacterial isolates, 30 had to decolorize the test dyes in respective media plates, 10 decolorized CR and 6 decolorized TB up to higher concentration (25 to 150ppm) of dyes (data were shown for only six isolates in table 2). Isolate no 4b shown decolorization up to 125 ppm of TB treated agar plate, and decolorization zone were observed 13 mm and 6mm at 75 ppm and 125ppm respectively, while isolates no. 4b, 5b and 6a shown more clear zone at 125 ppm of CR plate. Maximum decolorizing activities were observed in isolates 5b, and it decolorizes 100ppm of CR with forming 15 mm of zone (**Table 2**).

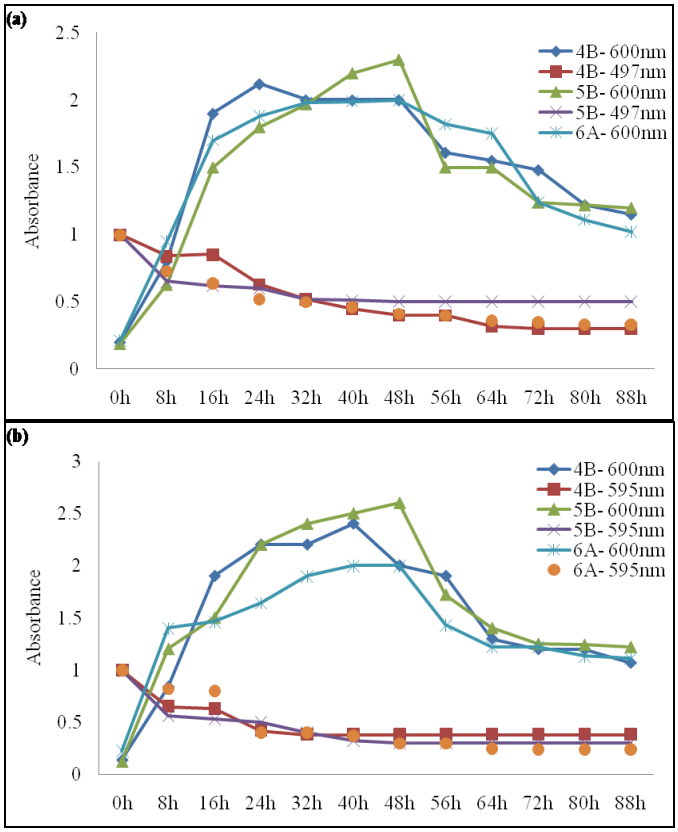

Fig. 1. Native bacterial growth and their dye decolorization activities upto 88h. (a) 50ppm of TB decolorization and (b) 100ppm of CR decolorization.

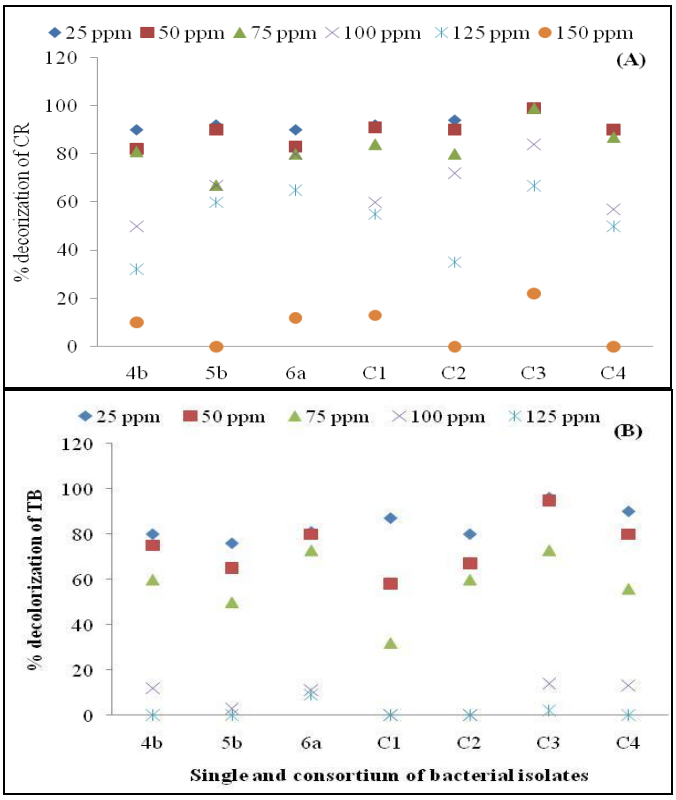

Fig. 2. Percent decolorization activities of potent native bacterial isolates (single as well consortium) of different dyes concentrations (A) TB and (B) CR. [Isolates 4b: *Pseudomonas* sp., 5b: *Bacillus* sp. and 6a : *Azatobacter* sp. and C1=4b+5b, C2=4b+6a, C3=5b+6a and C4=4b+5b+6a].

Bacterial colonies that showed a clear decolorization zone around the plates were measure by antibiotic zone scale (HiMedia), and those bacterial colonies were able to produced more than 12mm zone on the plates, reintroduced into 25 ml of freshly prepared enrichment broth media for further screening, and were incubated at 38 $\pm 2^0$C for 4 days at 100 rpm in incubator shaker, and decolorization pattern as well as bacterial growth both were observed (**Fig.1**) in dyes supplemented media broth medium upto 88h. In the present study, three bacterial strains namely 4b (*Pseudomonas* sp.), 5b (*Bacillus* sp.) and 6a (*Azatobacter* sp.) were capable to decolorize TB and CR. Sneha et al., [7] reported for decolorization of TB by *Pseudomonas putida* , In the present study, Isolate no 4b (*Pseudomonas* sp.), 5b (*Bacillus* sp.) and 6a (*Azatobacter* sp.) and their consortium [C1=4b+5b, C2=4b+6a, C3=5b+6a and C4=4b+5b+6a] showed significant decororization after 24h (**Fig. 2**). 94% decolorization of 100ppm of CR was observed in the consortium of C1 treatment followed by C4. Similar finding was reported [8] for CR decolorization by native pseudomonas bacterial strain along with bacterial consortium [19]. In the case of TB decolorization again consortium C3 showed best decolorization activities, and it gives 95% decolorization of 50 ppm of TB (**Fig. 2A**). Jaladoni-Buan [6] reported about monoculture as well as consortia of bacterial activities for CR decolorization. While decolorization trends of these bacterial isolates were shown different patterns for both CR and TB, but in the present study, native bacterial isolates decolorize more concentration of CR followed by TB. This might be due to different bacterial strains have different mechanism for azo-dye decolorization [3, 8]. Microscopic and biochemical identification of potent dye decolorizing bacterial isolates reviled isolate no 2a was *Alcaligen* sp., 4b was *Pseudomonas* sp., 5b was *Bacillus* sp., 6a was *Azatobacter* sp., 8c was *Lysinibacillus* sp. and 9d was *Alcaligen* sp (**Table 3**).

Table 3: Microscopic and biochemical identification of potent dye-decolorizing bacterial isolates.

Characteristic		Isolates number					
		2a	4b	5b	6c	8c	9d
Microscopic	Gram Stain	-	-	+	-	+	-
	Shape	SR	R	SR	SR	LR	SR
Salt tolerance	2%	+	+	+	+	+	+
	4%	+	-	+	+	+	+
	6%	-	-	-	-	+	-
	8%	-	-	-	-		-
Fermentation	Glucose	+	-	+	+	-	+
	Manitol	+	-	+	+	-	+
	Xylose	-	-	-	-	-	-
TSI slant	Slant	A	A	A	A	A	A
	But	A	A	A	A	A	A
	H_2S	-	-	-	-	-	-
	Gas	-	-	-	-	-	-
Hydrolysis	Starch	-	+	-	-	-	-
	Casein	ND	ND	ND	ND	ND	ND
	Gelatin	-	-	-	-	-	-
	Catalase	-	+	-	-	+	-
	NR	-	+	-	-	-	-
	MR	-	-	-	-	-	-
	VP	-	-	-	-	-	-
Temperature	25^0C	+	+	-	+	-	+
	35^0C	+	+	+	+	+	+
	45^0C	-	-	-	-	-	-

This indicate that sludge of carpet industries have diversity of bacterial strains with their coping behaviour against micro environment. The present finding indicates, these bacterial strains develop several mechanisms to survive against dye stuff. Therefore, we conclude native bacterial strains have better option to decolorize dye and it would be developing an eco-friendly technique for dye decolorization.

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Table 1: Physico-chemical, bacterial population analysis of sludge samples from carpet industries -Bhadohi (UP) India.

S.No	Sites	Sludge color	pH	ECe (dSm^{-1})	OC (%)	N (%)	PO_4 (%)	K_2O (%)	Bacterial population on different medias Cfu (10^4 g^{-1})		
									NA	KB	NMS
1	DPC	DB	7.63^{b}	5.69^{a}	6.20^{ab}	8.19^{a}	11.50^{b}	1.59^{ab}	56^{a}	31^{a}	36^{ab}
2	ICa	DB	7.90^{b}	9.47^{c}	4.40^{a}	11.54^{ab}	10.48^{b}	1.02^{a}	70^{b}	35^{a}	30^{a}
3	Dha	DB	8.71^{c}	6.60^{a}	6.70^{ab}	10.46^{b}	13.34^{c}	1.14^{a}	43^{a}	38^{a}	62^{c}
4	Ch	DB	8.85^{c}	8.13^{b}	6.40^{ab}	9.39^{a}	12.56^{b}	1.25^{ab}	100^{c}	35^{a}	78^{bc}
5	ICb	LB	6.91^{a}	8.60^{b}	5.90^{a}	12.50^{bc}	8.50^{a}	2.10^{c}	43^{a}	43^{b}	43^{ab}
6	ClRa	BBl	9.50^{d}	9.20^{c}	11.20^{c}	13.90^{c}	8.00^{a}	2.80^{bc}	87^{ab}	37^{a}	12^{d}
7	Ce	DB	8.21^{c}	6.50^{a}	8.20^{c}	12.60^{bc}	7.25^{a}	1.35^{ab}	65^{a}	27^{a}	43^{ab}
8	ARa	DB	8.63^{c}	7.90^{b}	10.90^{c}	13.60^{d}	6.90^{a}	3.21^{d}	78^{ab}	40^{a}	47^{ab}
9	DR	BBl	8.21^{c}	11.34^{cd}	12.60^{bc}	11.30^{bc}	9.50^{b}	1.65^{a}	66^{ab}	62^{bc}	43^{ab}
10	GpR	LB	7.22^{ab}	9.70^{bd}	11.26^{b}	5.40^{a}	9.10^{a}	1.50^{a}	73^{b}	38^{b}	42^{ab}
11	CL	DB	7.50^{a}	8.20^{b}	8.90^{d}	4.20^{a}	10.25^{ab}	2.60^{ab}	51^{a}	35^{b}	49^{ab}
12	ClRb	DB	8.11^{b}	6.20^{a}	6.70^{a}	11.00^{b}	8.60^{ab}	3.15^{bc}	54^{a}	33^{ab}	35^{b}
13	ICc	DB	8.20^{b}	11.50^{cd}	5.60^{a}	3.50^{a}	6.20^{a}	2.16^{b}	89^{d}	39^{ab}	39^{b}
14	Dhb	DB	8.20^{b}	9.50^{c}	8.50^{b}	4.00^{a}	7.60^{a}	2.35^{b}	30^{a}	43^{bc}	40^{b}
15	ARb	DB	8.12^{b}	8.90^{b}	7.50^{a}	4.70^{a}	8.60^{b}	2.00^{b}	48^{a}	30^{a}	28^{ad}

Figures followed by different letters in a same line are significantly different at P<0.05%, n=10. **[ECe** (Electrical conductivity), **OC** (Organic carbon in %), **N** (N content in %), **PO_4** (PO_4 content in %), **K_2O** (K_2O content in %), **Cfu** (colony forming unit), **NA** (Nutrient Agar), **KB** (King's B), **DPC** (Dharhura Police Chauki), **ICa** (Indra Colony), **Dha** (Dharhura), **Ch** (Chauk), **ICb**(Indra Colony), **ClR** (Civil line Road), **Ce** (Chauk-east), **ARa** (Auraiya Road), **DR** (Dharhura Road), **GpR** (Gopigang Road), **CL**(Civil Line), **ClRb** (Civil Line Road), **ICc** (Indra Colony), **Dhb**(Dharhura) and **ARb**(Auraiya Road), **DB** (Dark brown), **BBl** (Brown black), **LB** (Light brown).]

Table 2: Dye-decolorization activity on Nutrient agar plate supplemented with different concentration of dyes, (diameter of clear zone measured by antibiotic zone scale after 48h. (incubation temperature: 38±20C)

Test dyes	DDBI	Diameter of clear zone (mm) in Nutrient agar plate supplemented with different concentrations of dyes					
		25 ppm	50 ppm	75 ppm	100 ppm	125 ppm	150 ppm
TB	2a	++	++	++	nd	nd	Nd
	4b	++++	+++	+++	++	+	Nd
	5b	++	++	++	nd	nd	Nd
	6a	++	++	++	++	nd	Nd
	8c	++	++	++	nd	nd	Nd
	9d	++	++	++	++	nd	Nd
CR	2a	++	++	++	++	nd	Nd
	4b	++	++	++	++	nd	nd
	5b	++++	++++	++++	++++	++	nd
	6a	++	++	++	++	nd	nd
	8c	++	++	++	nd	nd	nd
	9d	++	++	++	++	++	nd

Data are average of triplicate, of six potent decolorizing bacterial isolates only ++++ (15mm), +++ (13mm), ++ (12mm) and + (6mm), DDBI (Dye-decolorizing bacterial isolates), Trypan blue (TB) and Cango red (CR).

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