International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 599-604
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Dye Degrading Microbial Isolates from Textile Soil - A Monograph

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Received: 12 Mar 2019 / Accepted: 16 Apr 2019 / Published online: 1 Jul 2019
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Abstract
The present study isolated dye degrading bacterial strain from textile soil Tirupur, Tamilnadu. Totally 14 strains were isolated from the sample. Out of 14 bacterial isolates strain NGP10 was selected as potent bacteria which degraded both crystal violet and Safranin dye up to 10mg/100ml with 90.1% of decolourization. Strain NGP10 showed, good degradation in pH 7, temperature 28°C and 10% bacterial inoculums containing medium. The potent strain NGP10 was identified as *Bacillus* species.

Keywords
Crystal violet, dye degradation, effluent, optimization, Safranin.

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INTRODUCTION
The human population enhances and boost up the necessity for cloths and food at the outset. People are magnetized to vivid fabrics and eventually it raises the number of dying industries which are the cause for our rich colored outfits. Dyes can be made either naturally or synthetically. However synthetic dyes are largely used in the dying industries. Dyes are classified as acid dyes, basic dyes, direct dyes, mordant dyes, vat dyes, reactive dyes, disperse dyes, azoic dyes and sulphur dyes. Nevertheless, more than 50% dyes are azo dyes owing to their chemical stability and versatility [1]. Global dyestuff productions are estimated to be around 34 million tons per annum. Just about 8000 chemicals are used in various processes of textile manufacturing and printing industries. Azo dyes, which collectively with chromophore is responsible for their brilliant color [2]. Ingestion of such dyes will result in the formation of N-hydroxylamines by the intestinal microorganisms and will lead to the DNA damage [3, 4]. Heavy mordants such as chromium are used to increase the fastness of dye on fabric [5]. The water used in the dying process is higher and half the amount is turned into wastewater with the addition of dye as a result of the fabric dying.

The industrial dyes are turned in to river and ultimately to ocean. It is detrimental not only to aquatic ecosystem (flora and fauna) but also barely to human beings [6, 7]. The aquatic environment with dye pollution is a serious and challenging part of studies now-a-days because of the decrease in light penetration into water and affecting the photosynthetic process. Nearby soils also get affected by untreated effluent and it has a direct impact on fertile agricultural land. The risk factor is that the removal of color as it is designed as biodegradation as they must tolerate the

environmental conditions and to stay with a color for a longer period of time.

The top pollutants from dye industries are chromium, lead and cadmium. Among which Chromium is a notorious carcinogen, lead generates neurological damage in children and cardiovascular disease in adults [8]. The composition may vary in the wastewater according to the dyes and chemicals used in the dying industries. They are characterized by parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH, color and salinity [9, 10].

Physical methods are not effective to volatile substance rather they just move effluents to another phase. Biological methods are effective since they degrade a whole dye leaving no residues. Many microorganisms such as bacteria [11, 12, 13], fungi [14, 15, 16] and actinomycetes [17] are studied and proved to have the capability of dye degradation but they are only at laboratory level. Some of the textile dye degrading bacteria are *Staphylococcus hominis* [18], *Bacillus cereus* [19], *Bacillus subtilis* [20]. Considering the dye industry pollution as one of the major environmental pollution and threat to life of animals and plants, this part of study brings our attention to isolate the dye degrading microorganism from the industry effluents, it may give solution by removing the hazardous compound from water and land resources resulting in the reduction of pollution level in the environment.

MATERIALS AND METHODS

Sample collection

The dye soil samples were collected from different dying industries in, Tirupur, Tamil Nadu, India. The samples were named as, TR1, TR2, TR3, TR4 and so on. The soil samples were collected in sterile airtight polythene bags.

Enrichment of sample and Isolation of dye degrading bacteria from soil samples

The sample collected from the textile was screened for dye decolorizing bacterial strains by inoculating 10 g of soil into 250 ml Erlenmeyer flask containing 100 ml nutrient broth. The flasks were incubated at 37°C under shaking conditions (120 rpm). After 48 h of incubation, 1.0 ml of the culture broth was appropriately diluted and plated on Nutrient Agar containing dye.

In this method, 1 ml of sample was thoroughly mixed with 9 ml of sterile distilled water, and then it was serially diluted by following standard procedure up to concentration of 10-6. Then, 1 ml of serially diluted samples from each concentration 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 and purified from the plates. The well grown bacterial cultures used for further screening technique and stored at 4°C.

Screening of dye degrading bacterial isolates from effluents

Twelve morphologically distinct bacterial isolates were tested for their ability to degrade the textile dyes. The isolated bacterial strains were screened out by incubating them on 100 ml of nutrient agar medium with 10 ml of dye. The nutrient agar medium incubated at 37°C for 24 hrs. After the incubation, plates were observed for clear zone. The screened culture was transfer to agar slant and store 4°C for further study. Four morphologically distinct bacterial isolates showing more than 75% degradation of the added dyes. These efficient bacterial strains were selected for further studies.

Identification of selected isolates

The four selected dye degrading bacterial strains were named as NGB 1, NGB 2, NGB 3, and NGB 4 based on their dye degrading ability, and they were identified using morphological and biochemical properties for the standard protocol of Bergey's Manual [21]

Dye decolourization experiments

Dye decolorization experiments were carried out in three 250 ml Erlenmeyer flasks for five soil samples. Each flask containing 100 ml of Nutrient Broth with 15 ml of soil samples. The pH was adjusted to 7± 0.2. Then, the flasks were autoclaved at 121°C at 15 lbs pressure for 15 minutes. The autoclaved flasks were inoculated with 5 ml of bacterial inoculum of each isolates and bacterial consortium. The flasks were kept in mechanical shaker and incubated at 37ºC for 4 days. Samples were drawn at every 24 hours intervals for observation. About 10 ml of the dye solution was filtered and centrifuged at 5000 rpm for 20 minutes. Decolourization was assessed by measuring absorbance at 510 nm of the supernatant with the help of spectrophotometer at wavelength maxima (λ_m) of respective dye.

Decolourization assay

Decolorization assay was measured in the terms of percentage decolorization using spectrophotometer. The percentage decolorization was calculated from the following formula,

$$\% \text{ decolouration} = \frac{OD_{initial} - OD_{final} \times 100}{OD_{initial}}$$

Bacterial categories

The isolates were selected based on three criteria; ability to degrade the dyes efficiently (> 75%), rapidly (within 2 days) and also ability to degrade a wide variety of dyes. A total of 18 consortia were developed using combinations of four isolates. A loopful of the selected isolates was individually inoculated into NB for 24 hrs to form a bacterial crew. 10% (v/v) aliquots of the culture mix were then transferred into a 250 ml Erlenmeyer flask containing 100 ml of Nutrient broth with pH adjusted to pH 7 with 15 ml of dye soil samples and allowed to react in agitated and static conditions. The decolorization of the dye was determined as per the procedure proposed by Khadijah *et al.,* 2009

RESULT AND DISCUSSION

Selection of Meritorious strain

Among the 14 different colonies one strain (NGP10) with the potency to degrade both Crystal violet and Safranin dye up to 10mg/100ml was selected and used for further studies. Dye degradation activity of all the isolates was represented in table 1.

Table 1: Dye degradation activity of bacterial isolates

S. No	Strains	Zone of clearance in mm		Turbidometric measurement (570 nm)		
				OD values taken after 7 days of incubation		
		CV	SFN	BLANK	CV	SFN
1.	NGP1	2	1	0.134	0.109	0.118
2.	***NGP2***	***4***	***3.5***	***0.134***	***0.068***	***0.079***
3.	NGP3	1.5	1	0.134	0.124	0.128
4.	NGP4	3	3.5	0.134	0.087	0.080
5.	NGP5	0.5	0	0.134	0.132	0.134
6.	NGP6	0	0.5	0.134	0.134	0.130
7.	***NGP7***	***5***	***4***	***0.134***	***0.047***	***0.066***
8.	NGP8	0	1	0.134	0.134	0.120
9.	NGP9	1	1	0.134	0.121	0.123
10.	***NGP10***	***4.5***	***5.5***	***0.134***	***0.044***	***0.039***
11.	NGP11	0.5	1	0.134	0.128	0.121
12.	NGP12	2	1	0.134	0.111	0.119
13.	NGP13	0.5	1	0.134	0.130	0.128
14.	***NGP14***	***4***	***3***	***0.134***	***0.065***	***0.090***

CV-Crystal violet SFN-Safranin: Blank- Nutrient broth with dye only
≠ All the values are mean of triplicates

Degradation of Crystal violet and Safranin by shake flask method

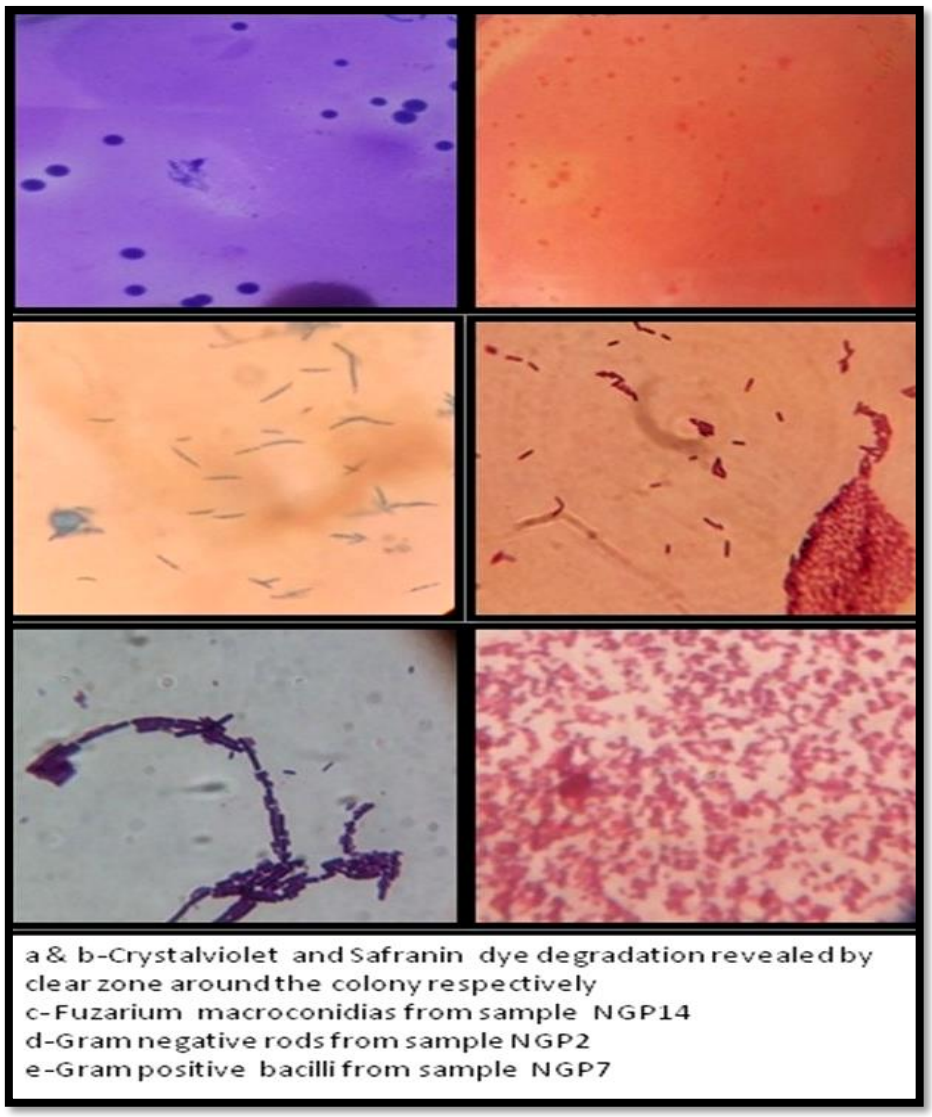
Suneja *et al.,* 2004 reported on the assay of malachite green decolorization in which the supernatant was analysed calorimetrically at 540 nm and in this present study the potent strain degrades 90.1% of decolorization was obtained at 0.10% of dye concentration within 36 hours.

Identification and characterization of bacteria isolated from textile dye effluent

The degradation of dye by biological methods is more effective and lower expensive of treatment and amenability to scale up easily are the merits of biological methods. The present study was focused on biodegradation of dye by using bacteria isolated from textile dye soil. Therefore, fourteen different bacterial isolates were isolate from 20 dye soil samples. Among the fourteen bacteria, the four bacteria are more effective against two dye solutions. 3 different dye degrading bacteria and a fungus of NGP2, NGP7, NGP10, and NGP14 identified as *Pseudomonas aeruginosa, Bacillus cereus, Micrococcus* sp. and *Fuzarium* sp. respectively. The characteristics of the identified bacterial isolates were furnished in Table - 2

Table 2: Morphological and Biochemical Characterization of Isolated Bacteria from textile soils

S.No	PROPERTIES		NGP2	NGP7	NGP10	NGP14
1.	Microscopic Observations	Gram's staining	G-ve rod	G+ve rod	G+ve Cocci	Fungi with macroconidia
		Motility	Motile	Motile	Non motile	NA
2.	IMViC Test		---+	--++	NA	NA
3.	TSI		K-/K-	A-/K-	A+/A-	NA
4.	Urease Test		-	-	+	NA
5.	Catalase Test		+	+	+	NA
6.	Oxidase Test		+	-	+	NA
7.	Nitrate Reduction test		+	Variable	-	NA
8.	Sugar Fermentation Test					NA
	Glucose		-	+	-	
	Lactose		-	-	-	
	Sucrose		-	variable	-	



a & b-Crystalviolet and Safranin dye degradation revealed by clear zone around the colony respectively
c-Fuzarium macroconidias from sample NGP14
d-Gram negative rods from sample NGP2
e-Gram positive bacilli from sample NGP7

Morphological identification and characterization of *Fusarium* species

The identification of *Fusarium* species is mainly based on distinctive characters of the shapes and sizes of macro- and microconidia, presence and absence of chlamydospores as well as colony appearances, pigmentations and growth rates on agar media [24]. Species identification was based on the morphological characteristics of single-spored isolates as described by Burgess *et al.,* 1994 and Leslie and Summerell 2006.

For microscopic characteristic, the isolates were cultured onto SDA agar for 2 to 4 weeks. Macro conidia were observed randomly, and the width and length were measured. Soil agar (SA) was used to enhance the formation of the chlamydospores [26]. For macroscopic observation, the cultural appearances (colony colour and pigmentations) were observed on potato dextrose agar (PDA). Colony colours and pigmentations were determined by using Methuen handbook of colour chart [27]

CONCLUSION

The present study concluded that, the isolated 3 different dye degrading bacteria and a fungus such as *Pseudomonas aeruginosa* (NGP2)*, Bacillus cereus* (NGP7)*, Micrococcus* sp. (NGP10) and *Fuzarium* sp. (NGP14) are potent strains for the treatment of textile dye effluent as it showed good degradation in all aspects. As it showed complete degradation of dye in the medium within 7days. Further, field study, other characterization such as structure elucidation proves their potential.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Host Institution Dr.N.G.P. Arts and Science College, Coimbatore for their moral and infrastructural support and Research Facilities by DBT – FIST and DBT – Star. We also thankful to our management, Principal, Deans of different portfolio, HOD of Microbiology and collogues for their valuable advices during article writing (Publication Communication No: DrNGPASC 2019-20/ BS002).

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