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Decolorization of Azo Dyes from Ranipet Textile Industrial Spent Wash Using Bacillus VIT SSG5

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ABSTRACT

In this study five soil samples were collected from different dye-industry locations. The bacterial micro flora from the five potential soil samples obtained were investigated for their dye-degrading activity. Out of 7 different bacterial isolates found in five soil samples, best dye-degrading activity was found in three isolates. Finally these three isolates were selected for further studies. An attempt was made to characterize the bacteria based on morphological and biochemical properties. This study demonstrates an efficient decolorization of dye by bacteria. The bacteria were able to decolorize dyes, which is very useful to degrade textile effluents. The analysis of degradation products by UV–Vis spectrophotometer showed formation of new and different products by bacteria, when incubated with dye for 72 hrs. Since the dye degradation process is enzyme dependent, the bacteria change the fate of metabolism to produce decolorized product. Out of the three bacterial strains showing good dye decolorization, the best strain was selected and identified to be as Bacillus VITSSG5 by flow charts of Bergey's Manual of Determinative Bacteriology. This study has provided a lead that soil bacteria can be used for minimizing environmental contamination by dyes used by textile industries.

Keywords: dye decolorization, strain improvement,bacteria,soil samples,degradation

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INTRODUCTION

The dye wastewaters are becoming more and more complex with the increasing diversity of dye products. Hence, the treatment of the waste water is becoming top priority to the researchers. The two major sources of dye-mediated pollution to the environment are the textile and dyestuff manufacturing industries [1]. With the increasing usage of the wide variety of dyes in these industries, the level of pollution from the effluents has become increasingly alarming. Dyes are of concern in wastewater treatment because of their color, bio-recalcitrance, and potential toxicity to animals and humans. Several physicochemical techniques have been used for treating wastewater containing dyes, but the methodologies have serious limitations and can lead to the generation of toxic by-products. Biodegradation using microorganisms is gaining importance as it is cost effective and environmentally friendly [2, 3]. Different taxonomic groups of bacteria have been reported for their ability to decolorize dye. Aerobic degradation of dyes has been reported by many investigators. The present work was undertaken in order to investigate the dye decolorizing potential of the isolated bacteria from waste disposal site of dye industry to decolorize various dyes[4,5] namely direct red 28, direct blue 1, direct green 6 and acid red 85.

MATERIALS AND METHODS

Chemicals and Dye

The medium components used in this study were from Hi-media Labs, Mumbai (India) and other chemicals used were of analytical grade. The dyes used in this study are Direct Red 28, Direct Blue1, Direct Green 6, Acid Red 85 [6].

Collection of Dye Waste Contaminated Soil Samples

Five soil samples were collected from waste disposal site of dye manufacturing plant and its surrounding areas [7, 8].

Isolation and Screening of Bacteria from Soil Samples [9, 10]

Samples were serially diluted to different concentrations. Two dilutions (2x and 4x) from each soil sample were taken. Each dilution was inoculated to dye amended nutrient agar media in triplicates by spread plate method. After incubation at 37 ˚C for 48 hours, plates were observed for bacterial colonies.

Purification of Bacterial Colonies

Each morphologically distinct colony appeared on Petri plate was further purified into single colony by repeated streaking on Petri plates containing nutrient agar media. After incubation at 37˚C for 48 hours, plates were observed for single bacterial colony.

Preparation of Dye Stock and Working Concentration [12]

In this study 4 dye samples were collected from commercial dye manufacturing Industries namely Direct Red 28, Direct Blue 1, Direct Green 6, Acid Red 85. These were used at different concentrations to amend culture media. A stock (10 g/l) from each dye was prepared in sterile water.

Decolorization Ability of Bacteria In Solid Media [11]

All the isolates were inoculated to dye amended media, incubated at 37 ˚C and and observed daily. The Decolorising activity was judged by the presence of clear zone surrounding the colonies. Based on decolorization activity, 7 colonies were selected. These isolates were inoculated to dye amended media again and finally 3 best isolates were selected.

Decolorization Ability of Bacteria In Liquid Media [13]

Decolorization of dye samples was studied in nutrient broth medium having 100ppm concentration of dyes. 0.25ml of seed culture was inoculated to 4.75ml of dye amended nutrient broth and kept for incubation in shaker. Three controls were kept, in which seed culture was not added. After 72 hours of incubation all samples were centrifuged at 10,000 rpm for 10 minutes and absorbance value was taken by UV-Vis spectrophotometer at different wavelengths according to the dyes.

To Isolate Bacteria Having High Degrading Activity

The isolated bacteria were inoculated to the dye amended nutrient broth and incubated for 72 hours at 37 ˚C. The dye degradation activities of 3 isolates were compared using spectrophotometer.

Dye Decolorization Study in Mixture of Dyes

Under this sterile water was taken and and a mixture of 8 dyes out of which 4 were known and 4 unknown were added along with the 10% seed culture. The effect of the bacteria on this mixture of dyes was observed daily and a decolorization of the dye mixture was seen.

Effect of Physical Parameters like pH, Temperature, Dye Concentration and Agitation on Dye Degrading Ability

Effect of pH

Isolate RR1 was inoculated to above different pH media and 5% of its seed culture was added to the dye amended different pH media. After incubation for 96 hrs, effect of pH was compared.

Effect of Temperature

Dye amended nutrient broth was inoculated with seed culture and incubated for 48 hrs at different temperatures of 28 ˚C, 37 ˚C and 45 ˚C. The effect of different temperatures was compared after the incubation for 96 hrs.

Effect of Dye Concentration

In this study 4 dye concentrations of 100ppm, 200ppm, 300ppm and 400ppm were made and 5% seed culture was added to each concentration after which they were incubated for 48 hrs after which the effect was compared using spectrophotometer.

Four working solution was prepared:

i. 50 µl stock + 5ml nutrient broth (100 ppm)
ii. 100 µl stock + 5ml nutrient broth (200 ppm)
iii. 150 µl stock + 5ml nutrient broth (300 ppm)
iv. 200 µl stock + 5ml nutrient broth (400 ppm)

From the above solutions we obtained working concentrations of 100 ppm,200 ppm,300 ppm,400 ppm.

Study of effect of agitation

In this study two sets of triplicates of 100ppm dye concentration were prepared and inoculated with 5% of seed culture. One set was kept on shaker (100 rpm) and other set was kept under static condition. After 96 hrs of incubation effect was compared using uv-spectrophotometer.

To Characterize the Member Bacteria of Isolates Based on Biochemical Profiles [15]

Gram's reaction, Citrate Utilization Test, Catalase Test, Indole Test, MRVP (Methyl Red Vogues Proskauer) Test, Triple Sugar Iron Agar Test, Starch hydrolysis test were done to characterize the bacterial isolates.

Strain Improvement of Selected Isolate Using Uv And Ethidium Bromide

Isolate RR1 was grown for 24 hrs in LB broth and exposed to UV light at interval of 5 minutes to induce mutagenesis. And also Isolate RR1 was grown for 24 hrs in LB broth prior to being treated with different concentration of EtBr for 60 minutes to induce mutagenesis [14].

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RESULTS AND DICUSSION

Isolation and Screening of Bacteria from Soil Samples

Soil Sample No.	Source	Types of Colony based on Morphology (No.)	Soil depth (cm)
1	Waste disposal site of dye plant, Ranipet, TN	RR1 and RR2	`5
2	Area near dye plant, Ranipet, TN	RR3	5
3	Area near dye plant, Ranipet, TN	RR4 and RR5	5
4	Area near dye plant, Ranipet, TN	RR6	5
5	Area near dye plant, Ranipet, TN	RR7	5

Table 1: Bacterial microflora isolated from different soil samples

Different bacteria were isolated from the soil samples which were collected from the dye contaminated sites. Some bacteria have got natural ability to degrade dyes by continuous exposure to dye polluted environment. These can be isolated and can be used to remove dye pollutant from environment. Table 1 describes Bacterial microflora isolated from different soil samples.(Table 1,fig 1)

Fig 1:Serial dilution of soil sample

Purification of Bacterial Colonies

Bacterial colonies obtained by isolation were purified by streaking on Petri plate having nutrient agar media. A single pure colony was obtained by this method for further study.(fig 2,fig 3)

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Fig 2. Streaked microbial colonies Streaked microbial colonies

Fig 3: Pure culture slant

The Dye Degrading Potential of the Selected Bacterial Strain

Dye decolorizing ability of selected isolates was investigated using spectrophotometer. ʎ max of each dye was determined by wave scanning in the spectrophotometer between range of 300 nm and 700 nm.

Dye Stock and Working Concentration

For decolorization activity different dye stocks were prepared. It was observed that at low concentration of dyes efficient dye decolorization occured. So, a low concentration (100ppm) is used to study decolorization effect.

Decolourization Ability Of Bacteria

It was observed decolorizing ability of different bacteria varied for different dyes involved. So, bacterial isolates having broad range of decolorization abilities were selected for further study. (Table 2)

Table 2: Quantitative determination of dye degrading activity by bacterial isolates from soil samples

Isolate	DR28	DB1	DG6	AC85
RR1	-0.699	-0.915	-0.665	-0.586
RR2	-0.587	-0.478	-0.215	-0.467
RR3	-0.447	-0.791	-0.225	-0.396
RR4	-0.540	-0.851	+0.306	+0.018
RR5	+0.021	-1.009	+0.330	+0.121
RR6	-0.541	-0.858	-0.222	-0.451
RR7	+0.178	-0.918	-0.269	-0.329

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Dye Decolorization Study in Liquid Media (Nutrient Broth)

Decolorization of dye samples direct red 28, direct blue 1, direct green 6 and acid red 85 was done by selected isolates and studied in dye amended nutrient broth for the quantitative analysis. Result showed that less amount of dye can be degraded more easily. As concentration of dye increased in the media decolorizing efficiency decreased.

Dye Decolourization Study at 24 Hr Interval.

Dye decolorization in liquid media was observed and the decolorization activity of consortium was studied using following formula:

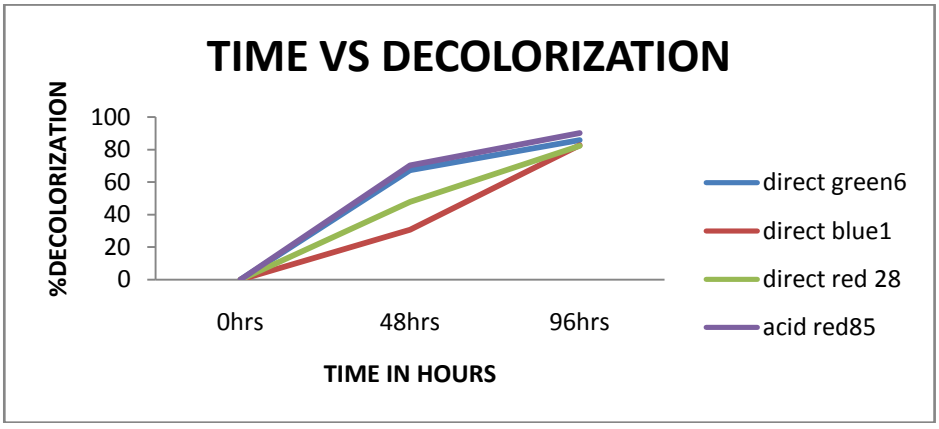
% Decolorization= (initial absorbance-final absorbance)/initial absorbance

(Table 3,fig 4)

Table 3:Dye decolorization in liquid media of all dyes

Color	Time(hrs)	S1	S2	S3	average	%decolorization
Direct green 6	0	1.047	1.058	1.087	1.064	0
	48	0.373	0.331	0.339	0.348	67.29
	96	0.169	0.146	0.138	0.151	85.81
Direct blue 1	0	0.899	0.898	1.007	0.935	0
	48	0.621	0.645	0.676	0.647	30.80
	96	0.149	0.150	0.186	0.162	82.67
Direct red28	0	0.876	0.858	0.886	0.873	0
	48	0.408	0.488	0.476	0.457	47.65
	96	0.181	0.136	0.136	0.156	82.13
Acid red85	0	1.547	1.526	1.538	1.537	0
	48	0.286	0.408	0.476	0.457	70.25
	96	0.160	0.157	0.422	0.153	90.04

Fig 4:



Three isolates were investigated for best dye decolorizing strain. RR1 was found to be the best strain among three isolates.(fig 5,fig 6,fig 7,fig 8)

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TO ISOLATE BACTERIA HAVING HIGH DYE DEGRADING ABILITY

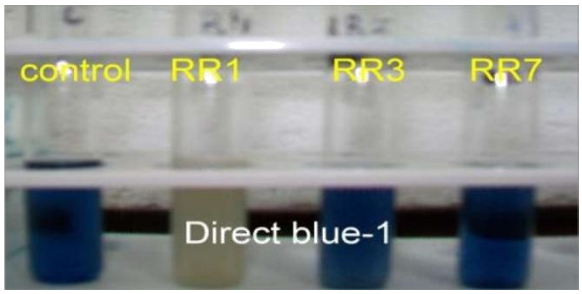

Fig 5: Decolorization of direct red 28 with selected isolate

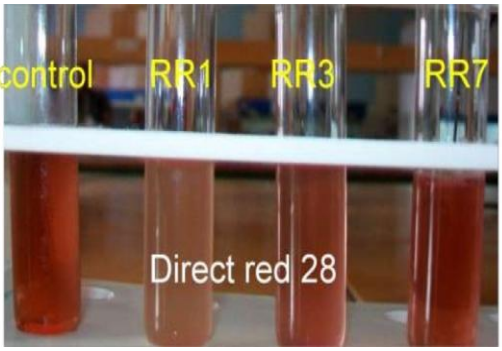

Fig 6: Decolorization of direct green 6 with selected isolate

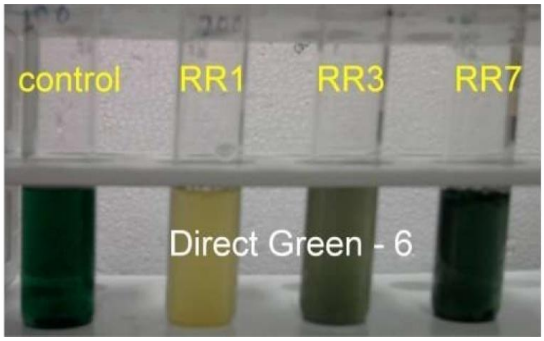

Fig 7: Decolorization of direct blue 1 with selected isolates

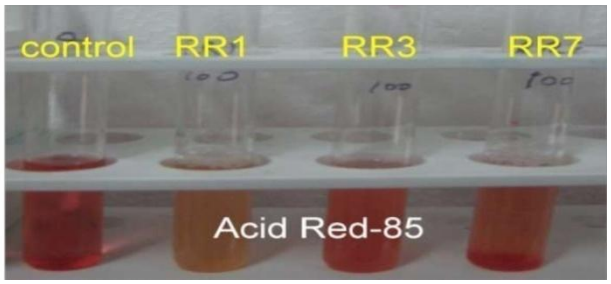

Fig 8: Decolorization of acid red 85 with selected isolates

Daily Decolorization of Mixtures

When a mixture of dye was inoculated with 10% RR1 culture it shows gradual decolorization. (fig 9,fig 10,fig 11,fig 12)

Fig 9:

Day 1

Day 2

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Fig 10:

Day 3

Day 4

Fig: 11

Day 5

Day 6

Day 7

Effect of Physical Parameters on Dye Degrading Ability

Dye degrading ability of the isolate was investigated with special reference to pH, temperature, dye concentration and agitation

Effect of pH. It was observed that best dye decolorization takes place at pH 7 with Isolate RR1. However dye decolorization was present in acidic and basic pH but here efficiency was found to be less.(fig 13, fig 14, Table 4)

Effect of Temperature

As seen that best dye decolorization takes place at temperature 37 ˚C with Isolate RR1. However dye decolorization is present at 28 ˚C and 45 ˚C but here less efficiency is found.(Table 5)

Effect of Dye Concentration

It was noticed that increasing concentration of dyes decreases the efficiency of decolorization by isolate RR1 .(Table 6,fig 15,fig 16,fig 17)

Effect of Agitation

Static condition dye decolorization was found here to be more effective.(Table 7)

Biochemical Tests

Biochemical test is done for three isolates RR1, RR2 and RR3. Among these three isolates RR1 is identified to be Bacillus vitssg5 according to Identification flow charts from Bergey's Manual of Determinative Bacteriology .(Table 8,fig 18,fig 19,fig 20,fig 21,fig 22,fig 23,fig 24,fig 25)

Strain Improvement of Selected Isolate Using UV And Ethidium Bromide

Strain improvement of the selected isolate RR1 was done and it was observed that fewer colonies appeared on Petri plates with increasing exposure to UV light. Similar result was observed with increasing concentration of Ethidium Bromide treatment with cultures. So, it can be concluded that colonies appearing in Petri plates with higher concentration of EtBr treatment and long exposure to UV light can easily survive in environment.

CONCLUSION

This work was carried out to screen, characterize tentatively identify the isolated bacteria from soil sample with best decolorizing ability. In this study bacteria were isolated from soil samples collected from waste disposal sites of dye and pigment industry and its surrounding areas. All isolates were investigated for their dye-degrading activity out of which seven isolates were found to have dye decolorizing activity with different efficiency. Out of seven different bacterial isolates found in five soil samples, best dye-degrading activity is found in isolate RR1. Two other strains RR3 and RR7 also have good decolorizing ability. But RR1 is found to be best isolated bacteria because it has broad range of decolorization. All studies were carried out in triplicates and their average was taken as final reading. An attempt was made to characterize the bacteria based on morphological and biochemical properties. The best strain was selected and identified to be as Bacillus VITSSG5 according to identification flow charts of Bergey's Manual of Determinative Bacteriology.

Dumping of dye stuff and dye waste water into the environment results in environmental pollution and medical problems. There is an urgent need for simple and cost-effective treatment methods for this problem. Microbial degradation and decolorization of the dyes gives us a hope to solve this problem as it is environment friendly, cost-effective and produces no harmful intermediates. Bacteria have an advantage over other microbes as they have a high growth rate and a high hydraulic retention time. Since various physicochemical parameters influence the decolorization performance, optimization of these is essential. Also further, to ensure the safety of the decolorized wastewater, studies should be conducted on the toxicity of the treated dye solution.

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