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DYE DEGRADATION BY MICROORGANISMS

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ABSTRACT

In the present study, an attempt was made to isolate, identify the dye degrading bacteria. A textile dye degrading bacterium was isolated from dye contaminated soil from Ichalkaranji. The dye degrading bacterium was identified as *Bacillus licheniformis* sp. F4 by 16S rRNA sequencing. The ability of this isolated bacterium for the efficient degradation of textile dye reactive golden yellow was evaluated. The effects of various physicochemical parameters such as pH, temperature, initial dye concentration, inoculum, carbon sources were examined for maximum degradation of textile dye reactive golden yellow. The isolated bacterial culture degraded reactive golden yellow (100 mg/l) by 80% after 24 hours of incubation period at optimum pH 7 and temperature 30^0C under shaking condition. A 15% (v/v) inoculum and 1% (w/v) glucose concentration were found to be optimum for degradation of textile dye reactive golden yellow. The biodegradation of reactive golden yellow was monitored by UV-Visible spectroscopy. The GC-MS analysis confirmed the biodegradation of textile dye reactive golden yellow. The result shows that the isolated bacterial culture has good potential in the degradation of textile dyes from textile waste water under aerobic conditions.

KEYWORDS: Textile Dye, Degradation, Microorganisms.

INTRODUCTION

Azo dyes comprise a diverse group of synthetic chemicals that are widely used by the textile, leather, food, cosmetics and paper industries.[1] The annual world production of azo dye is estimated to be around one million tons.[2] Azo dyes are considered as xenobiotics compounds that are very recalcitrant to biodegradation process.[3]

Some investigators reported that azo dyes and their metabolites are toxic, carcinogenic and mutagenic in nature which leads to the formation of tumors, cancers and allergies.[4]

Therefore treatment of the textile wastewater is essential before discharging the wastewater into a receiving water body.[5] Various physiochemical methods can be used for the removal of azo dyes from the wastewater. Some of these methods are effective but are quite expensive because they generate significant amounts of chemical sludge waste whose disposal in a secure landfill increases process cost.[6]

Degradation of dye by microorganisms is found to be an environmental friendly alternative. The ubiquitous nature of bacteria makes them invaluable tools in effluent treatment.[7]

The present study deals with the isolation and screening of indigenous bacterial strains, which had the potential to decolorize and degrade the various azo dyes and to study the effect of various physicochemical parameters on dye degradation.

MATERIALS AND METHODS

Chemicals: All the chemicals were of highest purity and of analytical grade. The textile dye reactive golden yellow was obtained from Radhamohan textile industry, Ichalkaranji, India.

Culture Media: The Mineral salt medium (MSM) used in the study contained (g/L): K_2HPO_4, 1.6; KH_2PO_4, 0.2; $(NH_4)SO_4$, 1.0; $MgSO_4.7H_2O$, 0.2; FeSO4.$7H_2O$, 0.01; NaCl, 0.1; $CaCl_2.2H_2O$, 0.02; glucose, 3 and yeast extract, 1.0.The pH the medium was adjusted to 7.5.The medium was sterilized at 121^0C 15 minutes.

Isolation and Screening of dye decolorizing and dye degrading bacteria

The soil samples were collected from dye contaminated soil, Ichalkaranji, were used for isolation and screening of dye decolorizing bacteria by enrichment culture technique using MSM amended with textile dye reactive golden yellow (50 mg/l). Dye containing media (100 ml) in 250 ml Erlenmeyer flask were inoculated with 10 ml soil suspension (10% w/v) and incubated in orbital shaker at 37^0C. Samples, which showed decolorization in liquid media, were repeatedly tested further by adding fresh dye containing medium till stable decolorization were obtained, showing consistent growth and decolorization in every successive transfer. An aliquot of 0.1 ml from the samples that showed consistent growth and decolorization in dye containing medium were transferred on MSM agar plates containing 50 mg/l respective textile dyes.

Identification of bacteria using 16S rRNA sequencing

Identification of the selected bacterial culture was performed by using 16S rRNA sequencing.

Decolorization assay by UV-Spectrophotometer

Absorbance of the supernatant withdrawn at different time intervals were measured at the maximum absorbance wavelength (λ max) at 600 nm for Reactive golden yellow, on Elico double beam spectrophotometer (SL-171). The percentage of decolorization was calculated from the difference between initial and final absorbance values. All the experiments were performed in triplicate.

Decolorization was expressed in terms of percentage of decolorization. This was calculated by using the following formula.

$$\% \text{ decolorization} = \frac{\text{Initial absorbance} - \text{final absorbance}}{\text{Initial absorbance}} \times 100$$

Effect of physicochemical parameters on dye degradation: Degradation was studied by using various carbon and nitrogen sources at different dye concentrations (50- 200 mg/l), at varying pH (1-14) and temperature (30, 35, 37, 40, 45 and 50^0C).

Analysis of the metabolites by GC/MS: After complete decolorization of textile dyes the culture broth was centrifuged at 7,000 rpm for 20 min to remove the cell mass. Culture supernatant containing the metabolites formed after degradation of textile dyes were extracted, using equal volume of ethyl acetate, dried over anhydrous Na2SO4 and concentrated in a rotary vacuum evaporator.

GC/MS analysis was carried out in Doctor's analytical laboratory, Pune. Rotary vacuum concentrated sample was dissolved in methanol and GC/MS analysis of metabolites formed after degradation of textile dyes was carried out using a QP 5000 mass spectrophotometer (Shimadzu). The ionization voltage was 70 eV. Gas chromatography was conducted in temperature programming mode with a Resteck column (0.25 mm × 30 mm; XTI 5). The initial column temperature was 40^0C for 4 min, which was increased linearly at 100^0C min-1_ 270^0C and held at 4 min. The temperature of injection port was 275^0C and GC/MS interface was maintained at 300^0C. The helium was used as carrier gas; flow rate was 1 ml min -1 and 30 min run time. The compounds were identified on the basis of mass spectra and using the NIST library stored in the computer software (version 1.10 beta Shimadzu) of the GC/MS.

RESULTS AND DISCUSSION

Isolation and Screening of dye degrading bacteria

Isolations of bacterial cultures from soil were carried out by the enrichment culture technique using MSM amended with Reactive golden yellow (50 mg/l). Out of 45 bacterial isolate, one bacterial isolate showed efficient decolorization ability on successive transfer. This bacterial isolate was selected for further study.

Identification of bacteria using 16S rRNA sequencing: Identification of bacterial isolate was done on the basis of 16S rRNA gene sequence. By the use of internal primers, 1.4 kb sequence of amplified 16S rRNA gene fragment was determined. The 16S rRNA sequence data showed that this strain had the highest homology (99 %) with *Bacillus licheniformis* (DQ520804.1). Therefore, this strain was named as *Bacillus licheniformis* sp F4. (Fig.1)

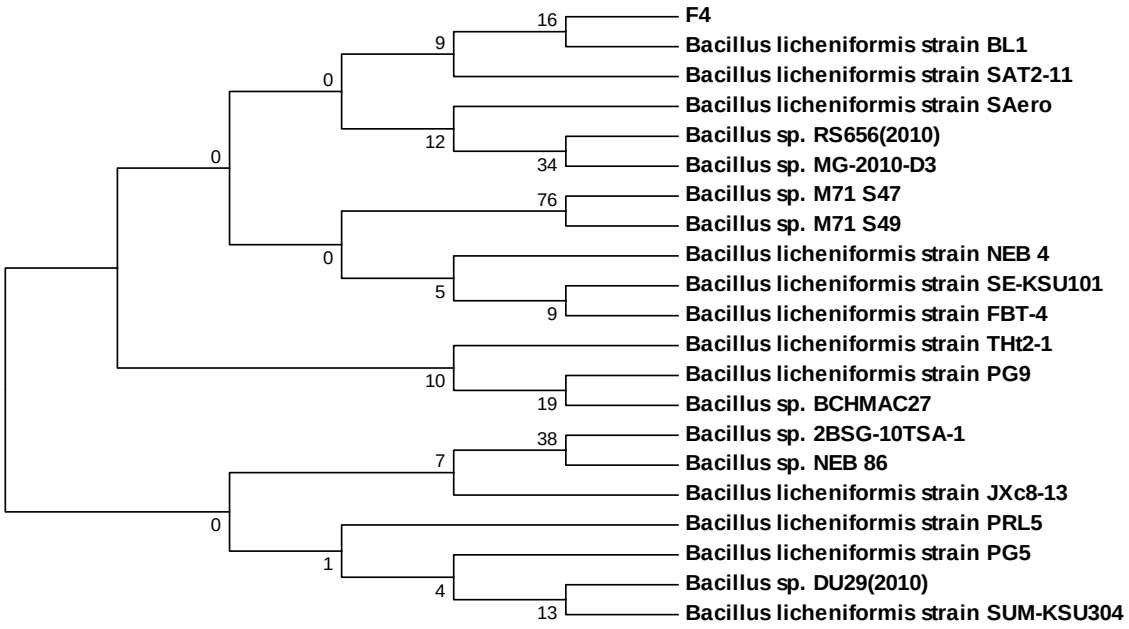


Fig. 1.

Effect of dye concentration on degradation of of textile dyes: The isolated bacterial strain *Bacillus licheniformis* sp. F4 was subjected to increasing concentrations of dyes from 50 to 300 mg/l in mineral salts medium at 30^0C within 24 hrs. The isolated bacteria F4 have shown 80.00% degradation 100 mg/l of reactive golden yellow dye. As the dye concentration increased there was decline in percentage degradation of all tested textile dyes by bacterial isolate F4 (Table 1). *Bacillus subtilis* was able to decolorize crystal violet dye up to 40 mg/l and the maximum decolorization rate was obtained when 15 mg/l crystal violet was applied.[8]

Table. 1: Effect of dye concentration on degradation of Reactive golden yellow by *Bacillus licheniformis sp*. F4.

Concentration of RGY 84 (mg/L)	% Degradation
50	75.81
100	80.00
150	64.18
200	60.00
250	56.05
300	51.11

Effect of pH on degradation of textile dyes: The effect of pH ranging from 1- 14 was tested on the percentage degradation of textile dyes. It was found pH 7.0 was found to be the optimum for maximum degradation of reactive reactive golden yellow (Table 2). Our isolated bacterium *Bacillus licheniformis* have exhibited 88.32 % degradation of textile dye reactive golden yellow at pH 7.0. *Bacillus subtilis* HM exhibited color removal capability of fast red over a wide range of pH (5-9), with optimum pH at 7.[9]

Table. 2: Effect of pH on degradation of Reactive golden yellow by *Bacillus licheniformis sp*. F4.

pH	% Degradation
1	15.26
3	38.22
5	63.55
6	75.16
7	88.32
8	70.10
9	63.00
10	51.05
12	33.66
14	10.52

Effect of temperature on degradation of textile dyes

We tested the effect of different temperatures on the degradation of textile dyes by our isolated *Bacillus licheniformis* sp. F4. It was found that 30^0C was the optimum temperature for bacterial isolate F4 for the maximum degradation of reactive golden yellow (83.00 %).[10] studied decolorization of reactive blacks by a bacterial strain *Enterobacter* sp. EC3.

Table. 3: Effect of temperature on degradation of Reactive golden yellow by *Bacillus licheniformis sp*. F4.

Temperature	% Degradation
30	83.00
35	80.23
37	82.33
40	69.14
45	61.07
50	44.60

Effect of different carbon sources on degradation of textile dyes

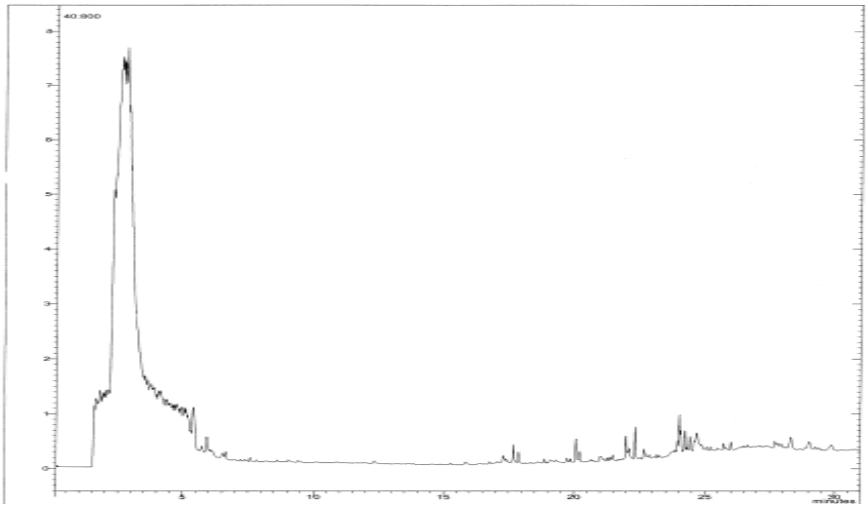
The effect of different carbon sources on degradation of textile dyes by our isolated bacterial culture was tested. It was found that 1% (w/v) concentration of glucose shown maximum degradation of reactive golden yellow-84 (81.05%) by isolated *Bacillus licheniformis* sp. F4. Moosvi *et al*. (2007) who reported that maximum decolorization (93 %) of Reactive violet 5R by bacterial consortium JW-2 was achieved at a glucose concentration of 1g/l.

Table. 4: Effect of different carbon sources on degradation of textile dye Reactive golden yellow by *Bacillus licheniformis* sp. F4

Carbon Sources	% Degradation
	Reactive golden yellow 84
Glucose	81.05
Lactose	72.40
Maltose	74.01
Sucrose	77.00
Fructose	71.92
Starch	60.41
Dextrose	79.00

Biodegradation analysis by GC-MS

GC-MS analysis was carried out to investigate the metabolites formed after the biodegradation of textile dyes. The metabolites formed after the degradation of reactive golden yellow by *Bacillus licheniformis* sp. were confirmed as hydroquinone, acetic acid octadecyl ester and dodecyl acrylate.



GC/MS Peak report of reactive golden yellow degraded metabolites by *Bacillus licheniformis* sp. F4.

CONCLUSION

The present study revealed the isolation and identification of bacterial strain as *Bacillus licheniformis* sp.F4 based on 16S rRNA sequencing. The results showed that the dye degradation is depend on carbon and nitrogen source, pH, temperature and initial dye concentration and inoculum concentration. Maximum dye degradation was observed at pH 7 and at 30^0c. *Bacillus licheniformis* sp.F4 have shown efficient degradation of reactive golden yellow (100 mg/l) by 80 %. GC-MS analysis confirmed the biodegradation of textile dyes by our isolated bacteria *Bacillus licheniformis* sp.F4. The results showed that our isolated bacterial strain have the potential application the treatment of textile wastewater.

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