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Research Article

Isolation and characterization dye degrading bacteria for detoxification of dark red 2B

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Abstract

In the present investigation, indigenous bacterial strains were isolated and capable for decolorization of direct red 2B azo dye commonly used in textile dying in Surat. A total of 13 bacterial isolates were isolated from effluent and soil sediment samples collected from chemically contaminated sites near the vicinity of Surat. These isolates were screened for their dye decolorization potential in Bushnell Hass minimal salt medium supplanted with 200 ppm of direct red 2B dye. Out of all of these two isolates namely JT1 and JT2 were selected for further investigation on the basis of maximum dye decolorization of 98.83% and 98.72%, respectively. The effect of pH, incubation temperature, carbon and nitrogen supplementationon dye decolorization by both isolates were studied. The cell free supernatant of these bacterial isolates was bioassayed on mung seeds for phytotoxicity studies. Good germination and shoot, root length of the plants were observed for degraded dye metabolite exposed seeds after comparing with the control. The isolates were identified as *Klebsiella spp.* for JT1 and *Staphylococcus spp.* for isolate JT2 on the basis of cultural and biochemical conditions.

INTRODUCTION

Environmental pollution is one of the major hazard to the modern world. Due to a large heap in industrialization, lots of chemicals along with dyes synthesized chemically and used in day to day life (Moorthi *et al.,* 2007). More than 10,000 commercial synthetic dyes of several classes have been produced worldwide with average annual production exceeding 2,80,000 tons (Jin *et al.*,2007). They are widely used in textile, paper, food, cosmetics and pharmaceutical industries with the textile industry being the largest consumer (Alalewi and Jiang, 2012).Among all synthetic dyes, azo dyes are the largest group of dyes used in textile industry due to the ease and cost effectiveness of their synthesis (Saratale *et al.*, 2011). Azo dyes are characterized by presence of one or more azo groups – N = N – and are the most common synthetic dyes released into the environment. During dying process, about 10-15% of dyes were lost in effluent (Zollinger *et al.*,1987). The presence of dye in aquatic ecosystem decreases rate of photosynthesis by impeding light penetration into deeper areas. Furthermore, the dyes or their degraded by-products may be toxic to aquatic flora and fauna (Talarposthi *et al.*, 2001). In addition to their visual effects, many azo dyes are toxic and in some cases they are carcinogenic to the humans as well as other animals (Saratale *et al.*, 2009).

Many physical treatmentslike ultrafiltration, reverse osmosis, ion exchange and absorption of dyes on various absorbents (activated carbon, peat, fly ash, and coal, wood chips, and corncob) (Choy *et al.*, 1999) and chemical methods like oxidation rections

photochemical oxidations and electrochemical treatments are available for the removal of dyes from textile effluent.Although widely applied, these methods are expensive and produces large amount of sludge which require safe disposal (Zille *et al.*, 2009). As an alternative, biological methods use different taxonomic groups of microbes such as bacteria, fungi, yeast and algae for the removal of azo dyes from the contaminated areas. Dye decolorization by fungi mainly involves adsorption rather than degradation and slow growth rate and low decolorization efficiency limits their use (Wang *et al.*,2009; Nigam *et al.*,1996). In contrast, bacteria can achieve a higher degree of degradation and even complete mineralization of dyes under optimum circumstances (Asad *et al.*, 2007; Gudmalwar and Kamble 2012; Bhoosreddy, 2014; Prasad 2014; Maheshwari and Shivagami, 2016).

Thus, the present study was aimed to isolate and characterize indigenous bacterial strain, capable of decolorizing Dark red 2B azo dye commonly used in textile industries of Surat, Gujarat, India. Additionally, effect of various physico-chemical parameters on decolorization of the dye by isolated bacterial strains was studied.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this study were of analytical grade and procured from Hi-media laboratories, Mumbai, India. The azo dye Dark red 2B used in this study was of industrial grade and purchased from local market of Surat, Gujarat, India.

Sample Collection and Enrichment

Samples were collected from chemically contaminated sites and enriched by inoculating 1 ml of sample in 100 ml sterile Bushnell Hass (BH) medium supplemented with 100 ppm of Dark red 2B in 250 ml erlenmeyer flask. Flasks were incubated at 30 °C at 100 rpm for 48 hr.

Isolation and Screening of Dye decolorizing bacteria

A loop full of enriched suspension was streaked on BH agar plates supplemented with 200 ppm dye and incubated at 30°C for 24 hr. Bacterial isolates showing zone of dye decolorization were then subjected to secondary screening. Screened isolates were inoculated in 100 ml BH medium supplemented with 200 ppm of dye. The flasks were incubated at 30°C at 100 rpm.

Selected bacterial isolates were streaked on nutrient agar and sent to Advanced Diagnostic Laboratory, Param doctor house, Lal Darvaja, Near Surat Railway Station, Surat, Gujarat for biochemical characterization. Biochemical tests were carried out on Phoenix Instrument, version: 6.01 A and Epi Center, version: V6.20A.

Measurement of percentage of Dye decolorization (Zimmermann*et al.*,1982)

The samples were collected and centrifuged at 10,000 rpm for 10 min at 4°C. Decolorization efficiency was analyzed by measuring the absorbance of culture supernatant at 530 nm using UV-VIS Spectrophotometer (Shimadzu-UV-3600 Plus). The decolorizing efficiency was expressed as percentage of decolorization:

Percentage of decolorization = [(Initial absorbance – Final Absorbance)/Initial absorbance] * 100.

Dye decolorization experiment

Selected bacterial isolates were inoculated in 100 ml BH medium supplemented with 200 ppm of Dark red 2B. The flasks were incubated at 30°C on rotary shaker at 100 rpm for 5 days. Samples were removed aseptically and dye decolorization was measured by the method described earlier.

Optimization of process parameters

Effect of pH and incubation temperature on dye decolorization

For pH optimization, initial pH of BH medium (with 200 ppm of dye) was adjusted to 5, 6, 7, 8 and 9 and decolorization assay was carried out. 1.5 ml of bacterial suspension was inoculated and incubated at 30°C at 100 rpm. The optimal temperature for dye decolorization differs greatly from one bacterium to another. 1.5 ml of bacterial suspension was inoculated in BH medium (with 200 ppm of dye) and incubated at various temperatures like 30°C, 37°C, 40°C and 50°C at 100 rpm. Samples were removed and checked for dye decolorization.

Effect of carbon and nitrogen supplementation on dye decolorization

Effect of additional carbon supplementation (glucose and sucrose) and nitrogen supplementation (ammonium chloride and urea) was tested for dye decolorization at various concentrations i.e. 0.2%, 0.5% and 1% (w/v). For this, 100 ml of BH medium (with 200 ppm of dye) were supplemented with different carbon and nitrogen sources in different concentration. 1.5 ml of bacterial suspension was inoculated and flasks were incubated at 37°C at 100 rpm. Samples were removed and checked for dye decolorization.

Phytotoxicity Studies (Phugare *et al.*, 2011)

Phytotoxicity was performed in order to assess the

toxicity of the untreated and treated dye to commo agricultural crops. The dye decolorization product was extracted with equal amount of ethyl acetate and recovered by evaporation of ethyl acetate phase and the components were dissolved in distilled water. Ten seeds of *Phaseolus mungo* (mung) plants were sowed into a plastic petri dish with daily watering of (5 ml) of 300 ppm dye solution (positive control) and extracted products (test sample). Negative control set was carried out using distilled water (daily 5-ml watering) at the same time. Germination and length of shoot and root were recorded after 7 days.

RESULTS AND DISCUSSION

Sample collection and Enrichment

Six different effluent and soil sediment samples were collected from three different contaminated sites namely Sachin GIDC (21°05'30.1"N 72°50'30.8"E), Pandesara Khadi (21°09'15.5"N 72°49'01.2"E), and Bamroli Khadi (21°07'56.6"N 72°48'43.3"E). Enrichment was done to promote the growth of bacterial populations in the samples.

Isolation and Screening of Dye decolorizing bacteria

13 different bacterial isolates showing zone of decolorization were isolated and were subjected to secondary screening. In secondary screening, selected isolated were inoculated in BH medium containing 200 ppm dye. After secondary screening, two bacterial isolates namely JT1 and JT2 were selected on the basis of maximum dye decolorization of 98.83% and 98.72%, respectively. On the basis of cultural and biochemical characteristics, selected isolates were identified as *Klebsiella spp.* for JT1 and *Staphylococcus spp.* for isolate JT2 (Data not shown).

Dye decolorization experiment

The results of dye decolorization in liquid medium showed that maximum dye decolorization up to 93.02 % was obtained for JT1 isolate as showed in the figure1.

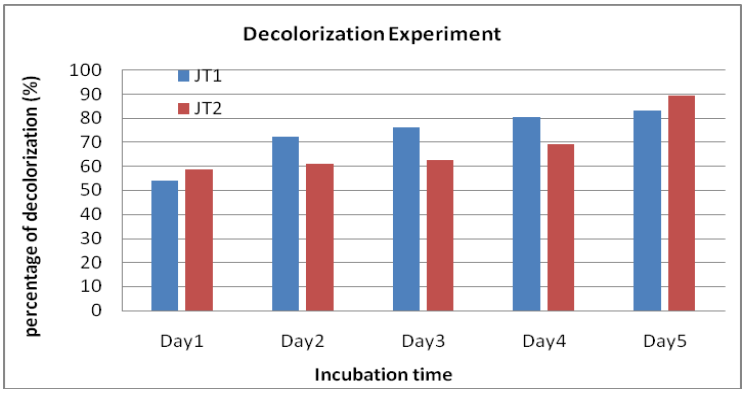

Fig. 1: Decolorization of direct red 2B by isolate JT1 and JT2.

Optimization of process parameters

Maximum dye decolorization of 75.62 % was obtained with isolate JT2 at pH 7 as shown in figure2.Similar results obtained by Chang *et al.*, (2000) who found that the decolorization was increased to 2.5 fold as the pH raised from 5 to 7.

For temperature optimization flasks were incubated at different temperature. Maximum dye decolorization of 87.35% was obtained with isolate JT1 at 37 °C of incubation temperature (Figure3). Our results were supported by Moosvi *et al.*, (2007). They found that bacterial consortium JW-2 showed maximum 93% decolorization of Reactive Violet 5R at 37°C. They also reported that further increase or decrease in temperature from optimum, decreases the decolorization rate.

The results of carbon supplementation study showed that maximum dye decolorization of 84.60% was obtained with bacterial isolate JT1at 1% glucose (Figure 4). Whereas sucrose was observed as poor carbon source showing only 65.38% of dye decolorization. Several reports are available for dye decolorization in presence of additional carbon source. Wang *et al.*, (2009) found 90% of dye decolorization in presence of glucose as additional carbon source for decolorization of Reactive Red 180 dye by *Citrobacter sp.* CK3. Carbon source provides energy for the growth and survival of microbes and act as electron donor which is important for the breakage of azo bond (Gonzalez *et al.*, 2009). The decolorization efficiency of isolates in presence of Ammonium chloride and urea as nitrogen sources was studied.

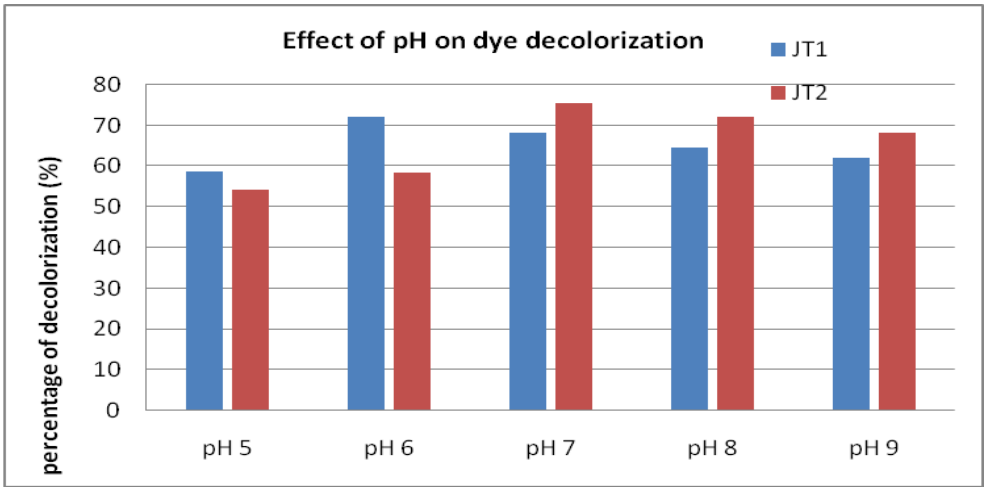

Figure 2. pH optimization for decolorization of direct red 2B.

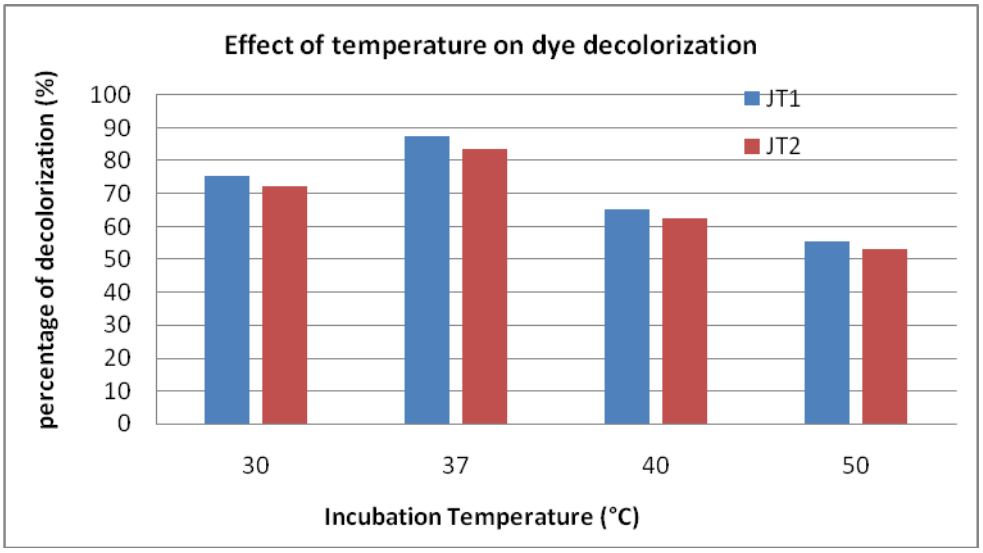

Figure 3. temperature optimization for decolorization of direct red 2B.

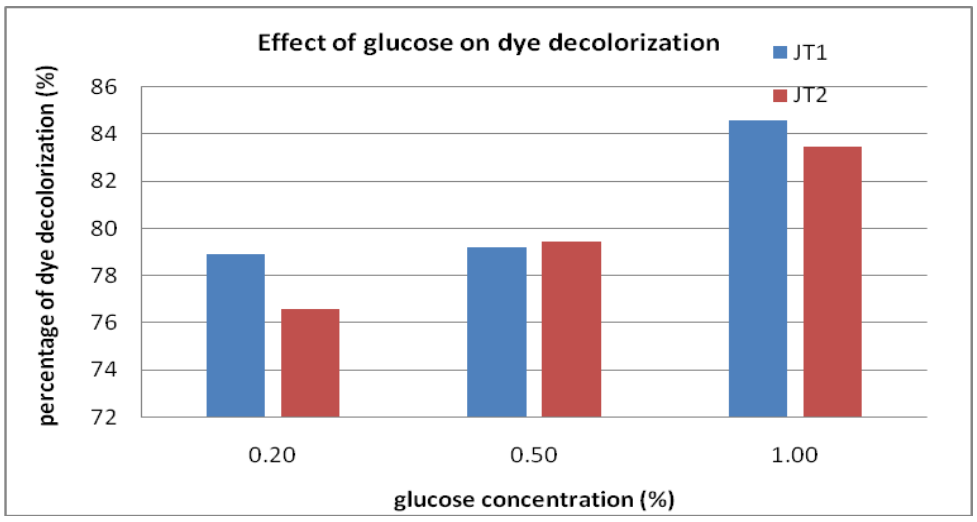

Figure 4. Effect of Glucose supplementation on decolorization of direct red 2B.

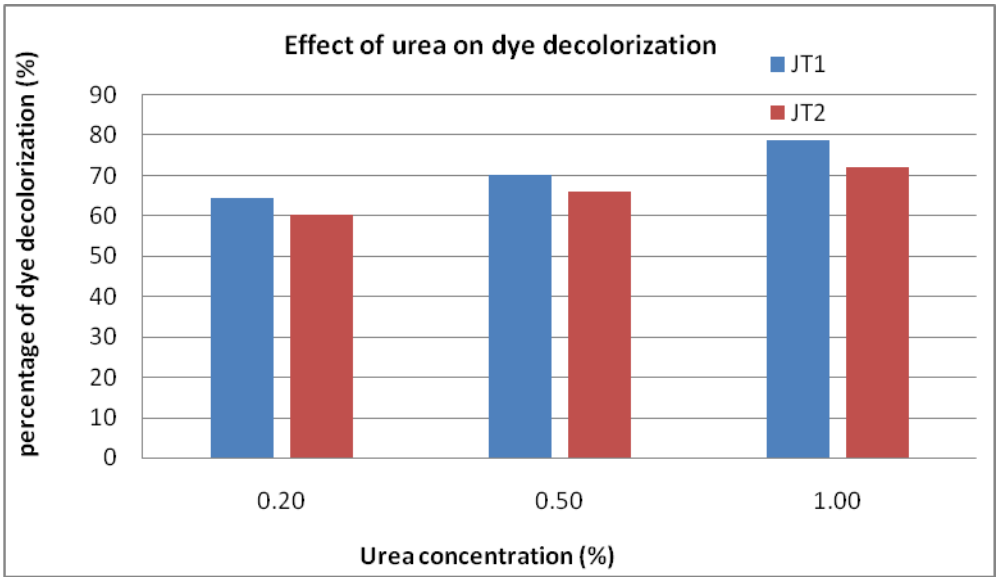

Figure 5. Effect of urea supplementation on decolorization of direct red 2B.

Phytotoxicity

Untreated or partially treated effluent may be disposed of in the water bodies and this water can be used for irrigation purpose. Thus it was found necessary to study phytotoxicity of the dye before and after degradation. Phytochemical studies showed that shoot and root lengths were affected in the presence of pure dye but it was less affected when tested with 300 ppm of extracted dye metabolites (Table1.). This indicates the detoxification of dark red 2B dye with isolate JT1. Extracted metabolites from dye decolorization broth of JT2 isolate did not showed any promising results for detoxification of dark red 2B thus are not shown here.

Table1. Phytotoxicity study of decolorization product of direct red 2B on *Phaseolus mungo*.

JT1	***Phaseolus mungo***		
	Water	**300 ppm pure dye**	**300 ppm extract**
Germination (%)	100	60	100
Plumule (cm)	16.9	5.2	14.6
Radicle (cm)	6.0	2.4	3.2

Gudmalwar and Kamble (2012) also found that there was no germination inhibition in *Vinga radiata* and *Sorghum vulgare* seeds when they were treated with the metabolites after complete decolorization of Reactive red 4E8Y5 dye by *Providencia spp.* and *Bacillus spp.* Similar results were also obtained by Ebency *et. al.*, 2013.

CONCLUSION

From this study it could be concluded, bacterial isolates were identified as *Klebsiella spp.* and *Staphylococcus spp.* form biochemical characterization and had potential of decolorizing textile dyes. Thus, they can be exploited for their potential of dye decolorization and can be used for bioremediation treatment of the industrial effluent.

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