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SHORT COMMENT

Decolourization of Textile Dye by Bacteria Isolated from Ganges Water Near Cossipore Region, Kolkata

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Abstract: Increasing environmental pollution due to improper disposal of industrial waste has created an upsurge for the development of ecofriendly and cost-effective methods of waste removal. Synthetic dyes discharged by textile industries, paper mills, plastic industries have been serving as potential pollutants to the soil and water bodies. Conventional physicochemical processes for dye decolorization are expensive and consequently generate secondary pollution due to the production of significant number of toxic derivatives. Hence, development of cheaper and environment friendly method has always drawn the attention of the researchers. The present investigation provides an insight on the role of bacteria in the detoxification of synthetic dyes. The study documented the role of nine bacterial isolates in decolorization of different dyes (malachite green, crystal violet, congo red, nigrosin and safranin). The isolates have shown best results in the degradation of malachite green and crystal violet. Presence of secretory amylase and laccase enzymes was also assessed biochemically.

Keywords: Bioremediation; Dye decolorization; Malachite Green; Crystal Violet; Congo Red

1. Introduction

Industrialization and rapid urbanization have become an inevitable mean of progress for a developing country in recent scenario but regrettably this imparts a huge threat to our environment. Textile industries, like many other industries continuously discharge industrial effluents containing synthetic dyes and toxic heavy metals into aquatic bodies, estuaries and landfills. These synthetic dyes are recalcitrant and serve as xenobiotic compounds (Pandey, Singh, & Iyengar, 2007). Inappropriate disposal of industrial effluents in natural resources like soil and water causes serious toxicity to the aquatic and terrestrial life. Approximately 1,00,000 different dyes are regularly used in different textile industries (A. Das & Mishra, 2017) which include azo, anthraquinone, thalocyanine, triphenylmethane and triarylmethane dyes. These dyes contain electron withdrawing groups which create electron deficiency in them. This aids difficulty in scavenging the dyes by the natural processes and the leftover residues cause serious deterioration of soil, water and other habitats by causing toxicity and carcinogenicity (Kaur et al., 2010). Hence, removal of these potential pollutants has become an exigency to restore the ecological niche of a habitat. Bioremediation or phytoremediation both have been proved to be better means over the conventional processes as they are cost effective and do not render toxicity. Physicochemical dye removal processes generally utilize filtration, coagulation, use of activated carbon, flocculation etc. all of which are very expensive and produces wastes that (Olukanni, Osuntoki, & Gbenle, 2006; Verma, Dash, & Bhunia, 2012) may also lead to secondary level of pollution (Shah, 2013). Dye decolourization by microorganisms have been extensively demonstrated in past few decades and showed considerable potential in bioremediation process in an eco-friendly manner (Kalyani, Telke, Dhanve, & Jadav, 2009). As evidenced from a number of

studies, bacteria, yeast, fungi produce various extracellular enzymes like laccase, lignin peroxidase, azo reductase, heme-peroxidase, manganese peroxidase exhibiting dye decolourization ability (Das et al., 2019). A number of studies have established the role of laccase in microbial dye decolourization process (Mani et al., 2019). Bacteria and fungi utilize these enzymes for the reduction of the dyes to get nutrient and energy source thereby converting the dyes into non-toxic forms (Pandey et al., 2007). The present study encompasses the role of microorganisms in decolourization of textile dyes. For this purpose, bacteria were isolated from Ganges water near the industrial region of Cossipore, Kolkata and bacterial isolates were used to study bio-decolourization of five different dyes namely Malachite Green, Crystal Violet, Congo Red, Safranin and Nigrosin. The study is aimed at determining an alternative to the conventional dye degradation method.

2. Experimental

Materials/Chemicals Details

Dyes malachite green, crystal violet, congo red, nigrosin and safranin were procured from E-Merck. Bacteriological media were purchased from Himedia. All reagents and media components were of highest purity.

Sample Collection and Initial Screening Samples of Ganges water (GW) were collected from industrial belt of Cossipore locality, Kolkata. Samples were serially diluted, plated on nutrient agar medium (pH 7.0) and incubated at 37°C. Isolation of potential strains was performed in triplicates. Isolates were screened for dye decolourization ability and out of several isolates only nine isolates showed the ability to decolourize dyes and hence these nine isolates were used for further studies.

2.1. Morphological and biochemical characterization of bacterial isolates

Morphological characterization of nine bacterial isolates was done using Gram staining and biochemical characterization was performed by IMViC test, starch hydrolysis and utilization of carbohydrates (lactose, fructose and dextrose).

2.2. Dye Decolourization Assay

Dye decolourization ability of nine bacterial isolates was carried out following the standardized method described earlier by our group (Ghatak & Das, 2018). Five different dyes (malachite green, crystal violet, congo red, nigrosin and safranin) were used for this purpose. Dye decolourization was assessed by the decrease in absorbance at respective wavelengths of the dyes. Inoculums (1 ml) of different bacterial isolates were separately given in 10 ml nutrient broth (pH 7.0) and were grown overnight at 37°C. The following day, dyes (1 ml in each tube) were added in each set at a concentration of 50 ppm and were again incubated at 37°C. Initial absorbance ('0' hr reading) was taken immediately after the addition of the dyes. Decolourization of the dyes was observed within 4 hrs. of incubation. Followed by incubation, 2 ml of culture was collected from each set and cells were removed by centrifugation and the supernatant was directly (without further dilution) used for taking absorbances at respective wavelengths. The percentage of dye decolourization was estimated using the following formula:

Percentage of Decolourization = $\frac{[(Initial\ absorbance - Final\ absorbance)]}{Initial\ Absorbance} \times 100$

All the experiments were repeated thrice and the data in the graph is represented as Mean $\pm$ SE.

Assay of Amylase and Laccase

Amylase assay was carried out by conventional method (Bernfeld, 1955) and laccase assay was performed using the method described earlier by Das and Ghatak, 2019. All the assays were performed in triplicate and results are represented as Mean $\pm$ SE.

3. Results and Discussion

3.1. Sample Collection and Initial Screening

Total 15 different bacterial isolates were screened for their dye decolourization ability out of which only nine isolates showed dye decolourization and hence further studies were performed with those nine bacterial isolates. These isolates were designated as GW and numbered from 1 to 9.

3.2. Morphological and Biochemical Characterization of Bacterial Isolates

Samples were screened by using nutrient agar medium and discrete isolated colonies were taken for morphological study. Morphological analysis showed that all nine isolates were gram positive in nature and either rod shaped or cocci. Other than GW1 all the isolates were found to be indole negative. GW2, GW6 and GW7 showed positive methyl red test while others were found to be negative. All isolates were negative for VP test except GW8 and GW9. Positive citrate test was observed only for GW1, GW2, GW4 and GW5. Interestingly, GW1 and GW6 showed starch hydrolysis ability and hence these two were further used for in vivo amylase assay. However, the strains did not ferment lactose, fructose or dextrose. Biodegradability is considered as the most cost-effective methods hence substantial efforts have been made by different groups of researchers to employ microorganisms for this purpose (Rebekah & Sharphudhin, 2016) whose prerequisite is to characterize particular strains. Results of biochemical and morphological characterizations are represented in Table-1.

3.3. Dye Decolourization Assay

Dye decolourization ability of nine bacterial isolates was studied based on the decolourization of five different dyes which are commonly used in textile and other industries (Durve, Gupta, & Naphade, 2012; Kumar & Saravanan, 2015). According to our earlier study (Ghatak & Das, 2018), the optimum concentration for the dyes were selected to be 50 ppm for each of the dyes and dye decolourization was represented in percent decolourization. Absorbances were recorded at 619 nm for malachite green, 586 nm for crystal violet, 495 nm for congo red, 570 nm for nigrosin and 515 nm for safranin. Among all the dyes, highest decolourization was observed for malachite green and crystal violet for all the isolates which could be correlated to an earlier study by (Das et al., 2019) . GW1, GW2, GW6, GW7, GW8 and GW9 all showed substantial decolourization of malachite green, however; the best result was documented for GW7 with a decolourization ability of 93.54%. Same result was also observed for crystal violet with GW7 showing the highest (89.4%) dye decolourization. In case of congo red and nigrosin, the isolated strains did not show marked decolourization effect; however, GW1 and GW8 showed 41.66% and 37.26% decolourization respectively for congo red and GW8 and GW9 showed 41.53% and 45.04% decolourization respectively for nigrosin. On the other hand, for safranin GW6 was found to be most effective (57.15%). Bacterial reduction of dyes often is associated with the activities of different enzymes but the difference in mechanism of metabolism among different bacteria could affect the efficiency of decolourization (Singh, Singh, & Singh, 2015). As GW1 and GW6 showed starch hydrolysis ability so they were screened for the presence of secretory amylase. The assay was performed at three different time points (24 hrs, 48 hrs and 72 hrs) and enzyme activity was highest at 72 hrs. GW6 gave better result (47.5 nmole/min/ml) than GW1 (16.1 nmole/min/ml) at 72 hrs (Figure 2A). Different scientific works established the role of laccase in biodecolourization of dyes (Singh et al., 2015). We observed that GW1, GW3, GW4, GW8 and GW9 all showed presence of secretory laccase with increase in activity on longer incubation. GW9 recorded highest activity with a value of 16.34 nmole/min/ml at 72 hrs (Figure 2B). It is of utmost importance to investigate the exact metabolic mechanism of these enzymes secreted by dye decolourizing bacteria to ensure the effectiveness of this method in a non-toxic manner.

Table 1. Morphological and biochemical characterization of bacterial isolates

Strains	Gram character	Indole	Methyl Red	Voges - Proskauer	Citrate	Starch hydrolysis	Utilization of Lactose/Fructose/Dextrose
GW1	Gram positive, short rods	+	-	_	+	+	_
GW2	Gram positive, coccus	_	+	_	+	_	_
GW3	Gram positive, coccus	_	_	_	_	_	_
GW4	Gram positive, short rods	_	_	_	+	_	_
GW5	Gram positive, coccus	_	_	_	+	_	_
GW6	Gram positive, rods	_	+	_	_	+	_
GW7	Gram positive, coccus	_	+	_	_	_	_
GW8	Gram positive, rods	_	_	+	_	_	_
GW9	Gram positive, rods	_	_	-	_	_	_

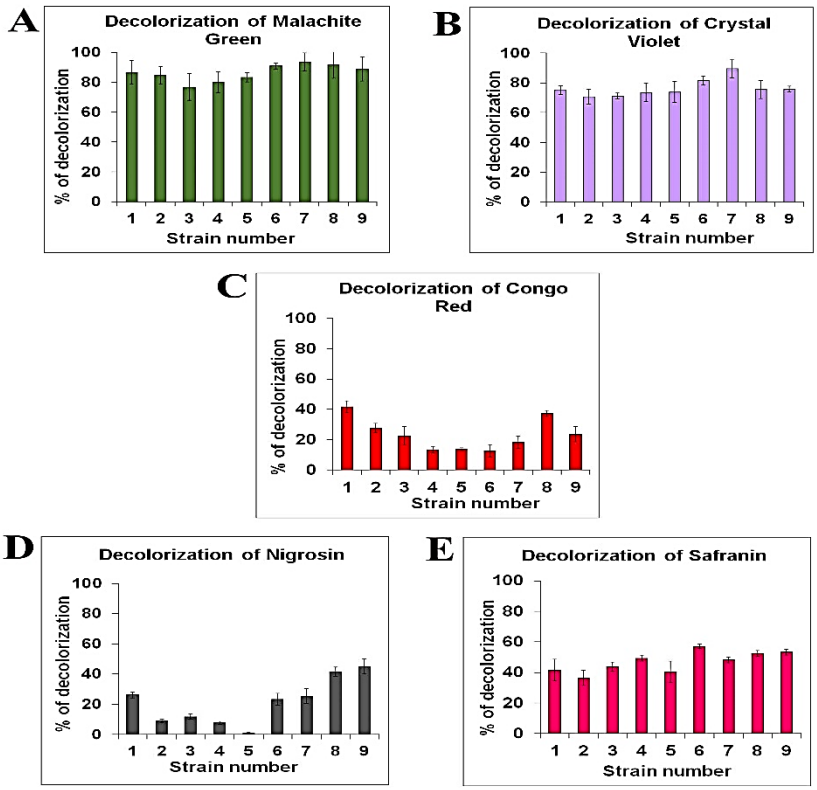

Figure 1. Dye decolourization assay of nine bacterial isolates using five different dyes namely malachite green, crystal violet, congo red, nigrosin and safranin. All the experiments were repeated thrice and the data in the graph is represented as Mean ± SE.

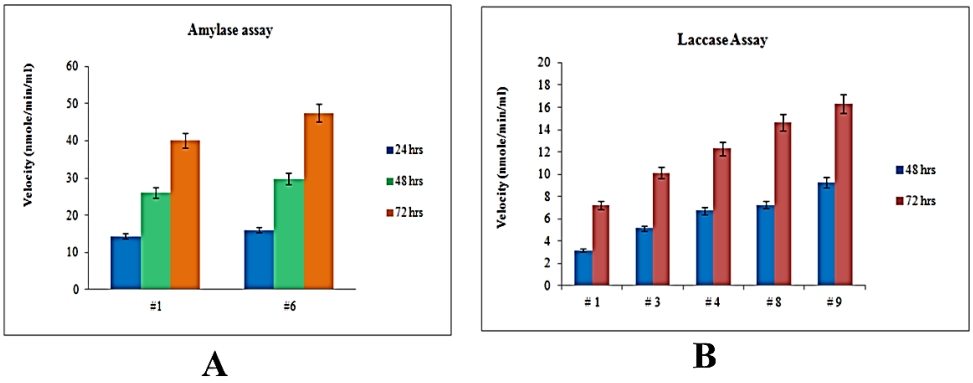

Figure 2. Assay of extracellular amylase and laccase enzymes of isolated bacterial strains. The results are represented as Mean ± SE of three replicates.

4. CONCLUSION

Bioremediation using bacteria have been practiced for decades by researchers for removal of toxic wastes generated by different industries. Synthetic dyes continue to be the potential pollutants of our natural environment. However, till date exact mechanism for biological decolourization of dyes has not been elucidated. Our study provides an insight for the role of bacteria in dye removal process although screening at molecular level is very important to identify potential strains with high dye degradation ability.

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6. AUTHOR'S CONTRIBUTION

Conceived the plan: Anamika Ghatak. Performed the experiments: Suchismita Das, Anamika Ghatak, Shonima Talapatra Ghosh. Data analysis: Suchismita Das. Wrote the paper: Shonima Talapatra Ghosh. Authors have no competing financial interests.

7. CONFLICT OF INTEREST

The author declares no conflict of interest.

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