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Bioremediation Strategies for Textile Dyes: A Sustainable Approach to Toxicity Mitigation

KRITI BHANDARI, SHEETAL SHARMA[1], TAPAN KUMAR MUKHERJEE[2], RAJAT LALLER[3], RAVI RANJAN KUMAR[4] AND MUKESH KUMAR[5]*

Department of Biotechnology, Chandigarh School of Pharmacy, Chandigarh Group of Colleges, Landran, Mohali-140 307 (Punjab), India
**(e-mail: mukeshkumarswami2150@gmail.com; Mobile: 78766 37382)*

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ABSTRACT

Release of untreated effluents into the environment results in soil and water pollution and subsequently reduces the fertility rate of soil. Nature has variety of microbes and their enzymes which can efficiently convert toxic effluents and chemicals into less toxic or beneficial alternatives. Thus, in present study, a bacterial isolate (*Priestia flexa* CGC) has been isolated, characterized and evaluated for its dye degradation potential. The conditions were optimized and it was observed that *P. flexa* CGC when inoculated to M1 medium of pH 7.5 containing necessary minimal salts and supplemented with 3% dextrose and meat extract resulted in 92.1% reduction of methylene blue. Furthermore, the effect of treated and untreated dyes on seed germination was also evaluated and it was observed that after treatment seed germination increased. Thus, from present study, it was concluded that *P. flexa* CGC was an efficient candidate for degradation of toxic dyes to enhance seed germination.

Key words: Synthetic dyes, *Priestia flexa*, bioremediation, environment pollution

INTRODUCTION

Textile industry is the largest growing industry worldwide and plays a significant role in economic growth and development of any nation. China is the largest exporter of textile good followed by USA, Turkey, EU and India (Sudarshan *et al*., 2023). Modernization and increasing population are the two major factors that significantly influence the textile sector. The dyes are colourful chemicals used majorly in textiles to impart colour to clothes. It is estimated that about 27% of revenue is generated from textiles in India. The majority centres for these same are in Surat, Gujarat; Ludhiana, Punjab and Thirupur and Karur in Tamil Nadu. Rapid developments in the textile industry have led to high demand for synthetic dyes. According to a recent study, more than 100,000 dyes and pigments are produced worldwide (Sudarshan *et al*., 2023). In the biodegradation of textile effluent components, microbial enzymes like laccase, peroxidase and azoreductase are frequently employed. Because of their capacity to effectively break down complex dye molecules, these enzymes present promising substitutes for traditional dye removal techniques. Whereas azoreductase focuses on breaking down azo dyes, peroxidase has a wide substrate selectively and gentle reaction conditions. Contrarily, laccase is efficient in degrading phenolic substances (Sarkar *et al.,* 2017). The blue dye known as indigo was discovered in the mummy wrappings of Egyptian tombs around 4,000 years ago, marking the first documented usage of an

[1]Department of Zoology, School of Basic Sciences, Abhilashi University, Chail Chowk, Mandi-175 028 (Himachal Pradesh), India.
[2]Amity Institute of Biotechnology, Amity University, Major Arterial Road, Action Area II-36,37,38, Rajarhat, New town, Kolkata-700 135 (West Bengal), India.
[3]Department of Botany, Indira Gandhi National College, Ladwa (Dhanora), Kurukshetra-136 132 (Haryana), India.
[4]Department of School of Allied Health Sciences, Rayat Bahra University, Kharar, Mohali- 140103 (Punjab), India.
[5]Department of Sciences, Chandigarh School of Business, Chandigarh Group of Colleges, Jhanjeri, Mohali-140 307 (Punjab), India.

organic colorant. Globally over 100,000 dyes are available for purchase, and over 7107 tonnes of dye-related materials are produced annually. Synthetic organic dyes, such as reactive, processing and direct dyes, are increasingly widely used in the textile industry. They are quite sophisticated due to the wide range of chemicals and dyes that are utilized to try to create more appealing, popular tints of fabrics for a competitive market. Environmental concerns related to the manufacturing and use of dye stuffs have developed dramatically over the past 10 years, and they are without a doubt one of the main factors influencing the textile dye business today.

All colours obtained from plants, animals and minerals fall under the category of "natural dyes". Since most natural dyes are not very strong, they must be applied to textiles with the aid of mordant's, which are typically metallic salts that have a preference for both the fibre and the colouring material (Burkinshaw and Salihu, 2019). The textile processing industries are the main users of synthetic dyes, which are widely employed in a variety of sectors (Al-Tohamy *et al.,* 2022). As early as 1856, Perkin was the first to produce mauve, an organic colour manufactured by humans (Al-Tohamy *et al*., 2022). Thus, in present study, dye degrading bacteria were isolated from different soil samples collected from different habitats, and conditions were optimized to enhance degradation potential. Furthermore, its phytotoxic effect on seed germination was also evaluated. This provided an eco-friendly approach degradation of toxic textile effluent in a cost-effective way.

MATERIALS AND METHODS

Samples of soil and waste water from the textile sector were gathered from dumping areas of textile industries located in Saharanpur (Uttar Pradesh) and Ambala (Haryana).

Prior to performing the serial dilution of the soil and water samples, the agar plates were prepared. Subsequently, 100 µl of the serially diluted 10^{-4} and 10^{-5} were spread out over the agar plates. Once these were solidified for a whole day, the plates were incubated at 37ºC. The colonies were then isolated based on their morphology (physical traits).

The primary method of characterising the isolated bacterial colonies was streaking them on methylene blue agar plates and observing their morphological characteristics. After that, the plates were incubated at 37ºC for 24 h. After that, the colonies that caused the methylene blue dye to deteriorate were separated, and fresh plates were created and streaked in preparation for the next step. The bacterial isolates showed positive responses during primary screening were then screened for secondary or qualitative screening. The culture was aseptically inoculated into 50 ml of fresh nutrient broth and incubated for 24 h at 37ºC in the shaker incubator. After incubation, 2% inoculum was added to the minimal salt medium containing 0.005 g of methylene blue dye as substrate and incubated for 48 h at 37ºC in a shaker incubator (Dimri *et al.,* 2020). Isolates showing maximum degradation were morphologically characterized and stored at 4ºC until further use. Percentage of decolourization was calculated as:

% D decolourization = 100 [(Initial absorbance - Final absorbance) / (Initial absorbance)]

The isolates were further morphologically characterised based on growth pattern, colony morphology on nutrient agar, Gram's staining. Gram staining is a staining technique used to divide bacteria into two major categories: gram-positive and gram-negative bacteria.

Molecular identification of isolated was done using 16s rRNA technology.

The effects of culture conditions on bacterial growth, such as carbon supply, nitrogen source, incubation temperature, medium pH, etc. were investigated using the one-variable-at-a-time (OVAT) approach. Every experiment was run under normal circumstances in duplicates. Three different growth media (M1-M^{1}) were screened for the decolourization of methylene blue dye. Each medium was inoculated with 2% of 24 h old bacterial culture and incubated at 37ºC for 48 h (Dimri *et al.,* 2020).

Carbon is one of the building elements of life so carbon sources play an essential role in the growth of microbes in synthetic media. Six different carbon sources used were; dextrose, sugar, starch, maltose, CMC and sucrose. Thus, six different media's were prepared containing different carbon sources in each media with same nitrogen source and other components as K_2HPO_4, KH_2PO_4, $MgSO_4$, peptone, $FeCl_2$ and methylene blue dye. In every media, these carbon sources were

different. After inoculating the media with 2% of bacterial culture, the media were kept in an incubator shaker at 37ºC for 48 h. One of the main elements that form the foundation of all living beings on Earth is nitrogen. Various sources of nitrogen, both organic and inorganic, were used to examine the impact they had on the activity of degrading dyes. Beef, meat, yeast, sodium hydroxide, ammonium di-hydrogen orthophosphate and sodium chloride were the nitrogen sources that were chosen. As a result, six media's were made, each having a different source of nitrogen. K_2HPO_4, KH_2PO_4, $MgSO_4$, peptone, $FeCl_2$, dextrose and methylene blue dye were then added (Choi *et al*., 2020). 2% of bacterial culture was then added, and the media were then incubated for 48 h at 37ºC. In order to determine the range and ideal pH, a variety of pH values (pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) were used for making media. In order to degrade the dye, 3% dextrose and 3% meat extract were added to each media, while the other ingredients remained constant. Fifty ml of media and 1 ml of new culture were added to the flask (in triplicate) at each pH level. After 48 h of incubation at 37ºC, the samples were centrifuged and an absorbance was made at 664 nm (Choi *et al*., 2020).

The effect of different treated and untreated textile dyes and effluent on seed germination of *Vigna radiata* (green gram) and *Cicer arietinum* (chickpea) were evaluated. The seeds were subjected to germinate in sterile 10 cm Petri dishes layered with the cotton beds. Seeds were sterilized before transferring to the surface of cotton in the Petri dishes (5 seeds per plate). The phytotoxicity bioassay was evaluated using the seed germination techniques. This method involved irrigation of seeds with treated and untreated effluent, dye and tap water to see the resultant effect. The seeds were allowed to grow at room temperature for three days in dark as well as light conditions. The germination % was calculated as:

Germination (%) = 100 (Number of seeds germinated in treatment/Total number of seeds)

RESULTS AND DISCUSSION

Total 16 bacterial isolates were isolated and screened initially based on their growth pattern and appearances on nutrient agar plate. Out of the 16 bacterial isolates, three isolates showing zone of decolourization on methylene blue agar plate in primary screening were designated as CGC1, CGC2 and CGC3. The isolates were further evaluated for quantitative decolourization in secondary screening to select the maximum/hyper dye decolourizer screened. Out of selected three isolates, isolate CGC2 showed maximum 65% decolourization of methylene blue followed by CGC3 (59.4%) and least in case of CGC1 i.e. 40%. Thus, isolate CGC2 was streaked on fresh agar plates and stored at 4ºC until further processing. Morphological features and gram staining of isolate CGC2 are shown in Table 1.

Table 1. Morphological characterization of isolate

Morphological features of the colony	
Characteristics	Observation
Appearance	Creamish to pale yellow
Margin	Smooth
Surface	Smooth
Edge	Smooth
Colony size	Large
Arrangement	Random
Temperature	30±5°C
Cell characteristics	
Gram character	Variable
Shape	Rods

16s rRNA technology is most preferred method to characterize the isolate on molecular level. The highly conserved 16s DNA segment was isolated and sequenced using sanger sequencing. The FASTA format was compared to find the maximum similarity using BLAST and phylogenetic tree was constructed to know the relationship using MEGAX (Fig. 1). The isolate showed maximum similarity *Priestia flexa* sp. and thus identified as *Priestia flexa* CGC. A genus of rod-shaped, predominantly Gram-positive bacteria belonging to the Bacillaceae family of the *Bacillus* order is called *Priestia*. In recent years, there has been an increase in the utilization of 16S rRNA gene sequences to discover novel bacterial strains. *Priestia* sp. was identified as the bacterial isolate from the soil and textile effluent after using 16S rRNA gene sequence to characterise it. Therefore, the study found that the isolate had sufficient capacity to decolorize the azo dyes, demonstrating the simplicity and effectiveness of the genotyping approach employing the 16S rRNA gene sequence in strain identification. The isolate's capacity for

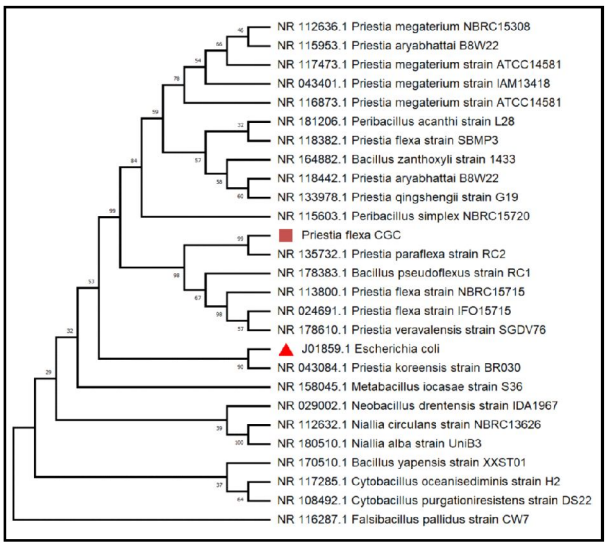

Fig. 1. Phylogenetic relationship of *Priestia flexa* CGC with closely related species.

bioremediation might thus be used to its advantage.

The growth medium plays an important role in the optimal growth of the bacteria and subsequent release of the products including enzymes. In order to optimize the best medium for growth and subsequent decolourization of dye, the isolate *P. flexa* CGC was grown in three different media (M1, M2 and M3). The maximum decolourization (78%) was observed when isolate was inoculated in M1 followed by medium M3 (Fig. 2). The activity was lowest in M2 (57%) might be due to unavailability of necessary nutrients and salts required for metabolic activity of isolate. M1 contained glucose and meat extract as carbon as well as nitrogen source, whereas M3 did not have any nitrogen source and M2 did not have carbon source. Since medium M1 emerged as the most appropriate medium for degrading activity, this growth medium was selected for future experiments.

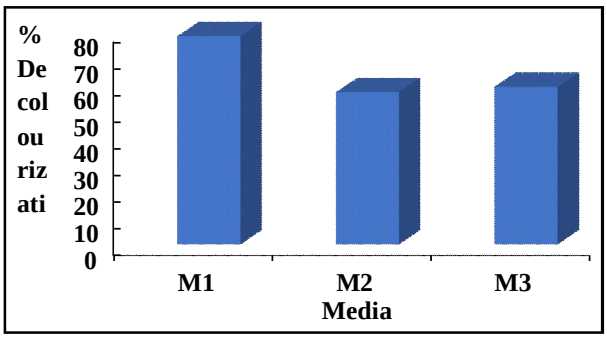

Fig. 2. Effect of different media constituents on decolourization of methylene blue.

After optimization of media components, resultant effect of different carbon sources on dye decolourization activity was also evaluated. For this, *P. flexa* CGC was inoculated in five different flasks consisting of M1 medium supplemented with different carbon sources (dextrose, starch, maltose, CMC and sucrose). 2% of 24 h old seed culture was inoculated in each media and incubated at 37ºC for 48 h in incubator shaker. After 48 h, the cells were harvested by centrifugation at 10000 RPM for 10 min and cell-free broth was analyzed spectrophotometrically at 664 nm. The maximum decolourization of 80.80% was observed when M1 medium was supplemented with dextrose as carbon source (Fig. 3). Decolourization potential was least in case of CMC might be due to its structural complexicity and recalcitrant nature.

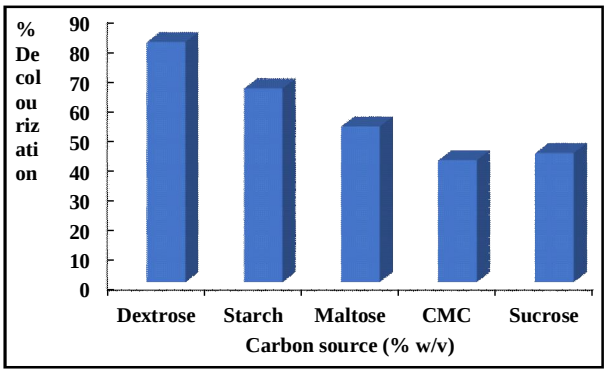

Fig. 3. Effect of different carbon sources on decolourization of methylene blue 4.5.

Like carbon source, nitrogen source also plays a significant role in enhancing the metabolic activity of microorganism and subsequently the production of enzyme. In order to determine the optimum nitrogen source for enhanced dye decolourization different organic (meat extract, yeast extract and beef extract) as well as inorganic (NaOH, $NH_4H_2PO_4$ and NaCl) nitrogen sources were used. The maximum decolourization of 85% was observed when M1 medium was supplemented with meat extract followed by beef extract and yeast extract (Fig. 4). Decolourization potential was least in case of $NH_4H_2PO_4$. The decolourization % was almost 50% less in case of inorganic nitrogen when compared with organic ones. This signifies that microorganisms prefer organic substrates more than inorganic.

After optimization of various nitrogen sources, resultant effect of different concentrations of nitrogen source on dye decolourization activity

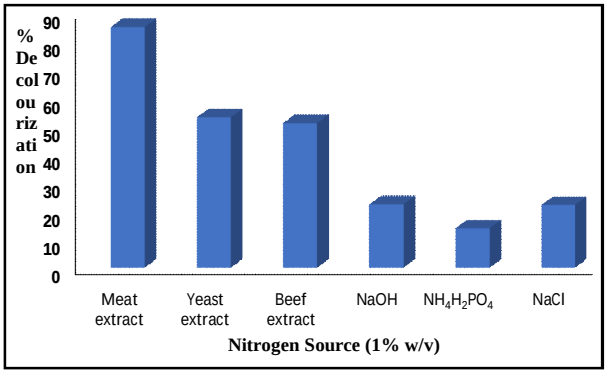

Fig. 4. Effect of different nitrogen sources on decolourization of methylene blue.

was also evaluated. For this, 2% of 24 h old culture of *P. flexa* CGC was inoculated into media containing different concentrations of meat extract and incubated at 37ºC for 48 h in incubator shaker. After 48 h, the cells were harvested by centrifugation at 10000 rpm for 10 min and cell-free broth was analyzed spectrophotometrically at 664 nm. The maximum decolourization of 90% was observed when M1 medium was supplemented with 3% (w/v) meat extract as carbon source. Decolourization potential was least in case of 0.5 concentrations.

Alteration in pH may also change the ionisation state of nutrient molecules and reduce their availability to microorganisms. After optimization of various carbon sources, resultant effect of different pH on dye decolourization activity was also evaluated. For this, *P. flexa* CGC was inoculated in eight different flasks consisting of M1 medium supplemented with different pH levels (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5). 2% of 24 h old seed culture was inoculated in each media and incubated at 37ºC for 48 h in incubator shaker. After 48 h, the cells were harvested by centrifugation at 10000 rpm for 10 min and cell-free broth was analyzed spectrophotometrically at 664 nm. The maximum decolourization of 92.10% was observed when M1 medium was supplemented with pH 7.5 and the least activity was observed at pH 5.5.

Time is a vital factor controlling the growth of microbes and subsequent production of metabolites. After optimization of different pH levels, resultant effect of incubation period on dye decolourization activity was also evaluated. For this, *P. flexa* CGC was inoculated in five different flasks consisting of M1 medium supplemented with 3% (w/v) carbon and nitrogen source while adjusting pH to 7.5. Then, 2% of 24 h old seed culture was inoculated in each media and incubated at 37ºC by varying incubation time in incubator shaker. The activity of all the cultures was noted when cells were harvested by centrifugation at 10000 rpm for 10 min and cell-free broth was analyzed spectrophotometrically at 664 nm. The maximum decolourization of 92.1% was observed when M1 medium was incubated for three days. It was least on day 1. In order to analyze the resultant effect of treated and untreated textile effluent on seed germination, two fast germinating plant species *Vigna radiata* and *Cicer arietinum* were selected. The surface sterilized seeds (5 seeds per plate) were subjected to germinate in sterile 10 cm Petri dishes layered with the cotton beds. The phytotoxicity bioassay was evaluated by measuring the length of radical and plumules every day for three successive days. At the end of the experiment, the rate of germination in tap water was 100% while in case of untreated dye and effluents 40-80% (Fig. 5, Table 2). Whereas an improvement of 20-40% in germination rate was observed when *P. flexa* CGC treated broth was used.

The radical length increases gradually as time increases. A maximum 03 cm growth was observed when tap water was used to moist *V. radiata* seeds to facilitate their germination followed by treated dye sample and least in case of untreated effluent. The radical length was approximately 70% less in case of untreated sample when comprised with control (tap water), which might be due to high concentration of toxic chemicals present in the effluent. Whereas after treatment (decolourization radical length increased from 0.9 to 2.2 cm).

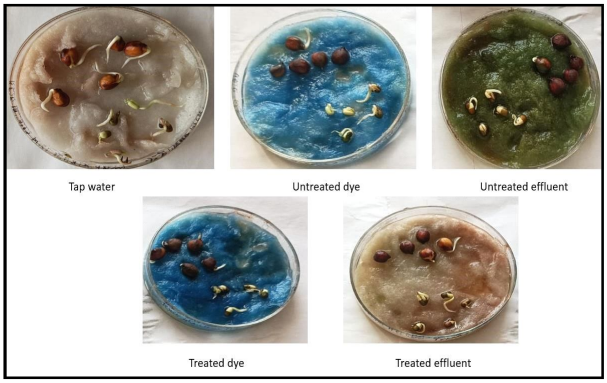

Fig. 5. Effect of treated and untreated textile dyes on seed germination.

Table 2. Toxicity test for selected plant species

Plant species	After 3 days of experiment	Tap water	Untreated effluent	Untreated dye	Treated effluent	Treated dye
V. radiata	No. of seeds germinated	05	03	03	04	04
	Radical length (cm)	03	0.9	01	2.3	1.9
	Germination (%)	100	60	60	80	80
C. arietinum	No. of seeds germinated	05	01	01	02	04
	Radical length (cm)	2.2	0.4	1	1.3	1.5
	Germination (%)	100	20	20	40	80

The growth pattern was almost similar in case of *Cicer arietinum*. This further confirmed the biodegradative potential of bacteria and strengthened its utility as potential candidate to reduce environment burden caused due to unmanaged discharge of textile effluent. Variety of microorganisms and their enzymes available in the environment are most commonly reported to degrade synthetic dyes. In the present work, dye degrading bacteria were isolated, screened and characterized for their morphological feature and used for their dye degradation potential. Further molecular characterization revealed it as a species of Bacillaceae family and genus *Priestia* and it was identified as *Priestia flexa* CGC. In a similar study, Kumar *et al*. (2023) also reported decolourization of dye from Bacillaceae sp. (Choi *et al*., 2020) screened *Bacillus cereus* for its degrading activity and observed that isolate degrades or decolourizes the dyes.

Optimization was done in order to provide most favourable conditions for growth and subsequent degradation of synthetic dyes. Different media constituents, carbon source, nitrogen source and external factors were optimized. Investigation of different nitrogen sources and carbon sources revealed meat extract and dextrose with 3% concentration showed maximum decolourization when medium of pH 7.5 was used and incubated at temperature 37ºC. Previously, Chanwala *et al.* (2019) also reported dextrose as the best carbon source for the decolourization of dyes using Planococcus sp. as it promotes rapid growth and enzyme activity for efficient degradation of textile dyes. The production temperature in present study was 37ºC, which was similar to the findings which had also reported maximum degradation of dye by Bacillaceae sp. at 37ºC. The temperature of 37ºC is used for the production of dye degrading bacteria because it optimizes their growth and metabolic activity. This temperature is close to the natural conditions for many bacteria, particularly those that are mesophilic. pH plays an important role in growth as well as on product formation (Yaseen and Scholz, 2019). Variation in external pH may directly affect the enzymatic activity by changing the metabolic functioning due to alteration of cytoplasmic pH and ionisation state of nutrient molecules. In the present study, maximum dye decolourization by *P. flexa* CGC was observed when pH of medium was 7.5. In a similar study, a maximum decolourization activity form was found in *Alcaligenes faecalis* sp. at pH 4-5 (Adane *et al.,* 2021). This also confirms that pH requirement varies with microbe to microbe. The working pH for textile process is in range 4.0-9.0, thus from the findings of present study, it can be concluded that the *P. flexa* CGC can act as potential candidate for its multifaceted applications in textile industry.

CONCLUSION

Textile dyes and effluent are menace to aquatic as well as human's life. The use of bioremediation to remediate textile effluents has become an affordable, effective and ecologically safe technique. Because bacteria and algae have well-developed mechanisms like oxidative and reductive enzymes that help cleave the chemical molecules of dye, they may be efficiently extracted from settings polluted by textile dyes for the purpose of bioremediation. Furthermore, microalgae and bacteria have the potential to treat dye-polluted wastewaters due to a number of additional advantages. These include shorter life cycles, a large surface area that allows for great biosorption, and the presence of chemical groups on their cell walls, which provide many sites for electrostatic adsorption. Thus, in the present study, a bacterial isolate (*Priestia flexa* CGC) was isolated, characterized and evaluated for its dye degradation potential.

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