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ORIGINAL ARTICLE

Broad spectrum Dye degradation by a single Bacterial Isolate: A case Study with Five Synthetic dyes

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ABSTRACT
Textile dyes are among the most persistent and hazardous pollutants released into industrial effluents, causing serious environmental and health concerns due to their complex aromatic structures and resistance to degradation. This study aimed to evaluate the dye-degrading potential of a single bacterial isolate against multiple classes of synthetic dyes. The bacterial isolate was tested for its ability to decolorize five different dyes. Methylene Blue, Crystal Violet, Congo Red, Malachite Green, and Reactive Black under controlled laboratory conditions. Decolorization efficiency was used as a measure of biodegradation capacity. The isolate demonstrated efficient decolorization across all dye types, indicating broad-spectrum enzymatic activity and strong adaptability to diverse dye structures. Its performance highlights the strain metabolic versatility. The bacterium exhibits promising potential for use in the bioremediation of dye-contaminated wastewater. Future studies focusing on process optimization and scale-up and could enable its practical application in industrial effluent treatment systems.
Keywords: *Textile dyes; Biodegradation; Bacterial isolate; Decolorization; Methylene Blue*

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INTRODUCTION

The textile industry is one of the largest consumers of synthetic dyes, producing vast quantities of colored wastewater that pose serious environmental threats. These dyes, originating from various natural and synthetic sources, are used to impart vibrant and lasting colors to fabrics. However, their complex chemical structures especially those of synthetic dyes like azo, anthraquinone, and triphenylmethane dyes make them highly resistant to degradation. As a result, untreated or poorly treated dye effluents released into water bodies lead to reduced light penetration, oxygen depletion, and toxicity to aquatic life [1].

To address this environmental concern, microbial degradation has emerged as a promising eco-friendly alternative to conventional physicochemical treatment methods [2]. Various microorganisms, including bacteria, fungi, and algae, possess the enzymatic machinery required to break down complex dye molecules into simpler, non-toxic compounds. These biological systems can adapt to different dye structures and environmental conditions, offering sustainable solutions for wastewater remediation. Economy, which is expanding quickly, is mostly dependent on the textile and ready-made clothing sectors [3]. Exporting textiles and clothing items is the nation's main source of foreign exchange profits. In 2016, China as the second-largest apparel producer. A good threat to living things is the release of contaminated water from industrial sources, which poses major environmental risks. Before industrial textile wastewater discharge [4]. Water pollution, where effluents come from dye-based industries like textile industries, serves as a principal source and is one of the most concerning environmental pollutants threatening our biodiversity. Generally, textile dyes are mostly alienated into azo, reactive, triphenyl methane, heterocyclic, polymeric compositions, etc. [5].

Azo dyes are the most appropriate for usage in the textile industry out of all the textile dyes. They withstand heat, light, water, bleach, and detergents quite well. Because of their distinctiveness and π-

conjugated azo bond property. It is projected that about 5-10 % of total dyes are released in textile wastewater streams during the dyeing process ultimately reaching the natural water bodies without treatments [6]. The majority of dyes and their byproducts, aromatic amines, are harmful to aquatic life, and some of them continue to have mutagenic and carcinogenic effects on both humans and other animals. The majority of dyes and their byproducts, aromatic amines, are harmful to aquatic life, and some of them can still cause cancer and mutagenesis in humans and other animals [7]. Additionally, the dye can disrupt the aquatic ecosystem by reducing light penetration, gas solubility, and phytoplankton photosynthesis. Moreover, the release of untreated dye effluent into drinkable water sources including lakes and rivers changed the pH level, increasing BOD, COD, and TOC evaluations [8].

Therefore, to reduce environmental pollution, textile industry effluents (dye-contamination) must be treated. Additionally, azo-dyes from textiles can occasionally be difficult to completely break down using standard physicochemical techniques such as activated carbon adsorption, flocculation, coagulation, and reverse osmosis [9]. Furthermore, there are several drawbacks to such conventional treatments, such as the use of excessive chemicals, higher energy costs, the production of massive amounts of sludge, and additional contamination [10]. This study explores the origins of textile dyes both natural and synthetic and evaluates the potential of different microorganisms in degrading these dyes. Understanding the interactions between dyes and microbial agents can contribute significantly to developing efficient bioremediation strategies for a cleaner and more sustainable textile industry [11, 12].

MATERIAL AND METHODS

Sample collection

Tirupur and Coimbatore provided the dye effluents for collection. Based on their location names, the samples were given the designations S1,S2,S3,S4 and S5 accordingly. Plastic cans were used to collect the effluent samples. The cans were washed with tap water and distilled water before the sample collection.

Isolation of the dye-degrading bacterial isolates from dye effluent

The serial dilution (Pour plate) approach was used to isolate the bacteria that were found in the textile dye effluent. This method involved thoroughly mixing 1 milliliter of the sample with 9 milliliters of sterile distilled water, followed by serial dilution according to usual protocol until the concentration reached 10-6.Following that, 1 milliliter of serially diluted samples from each concentration was put into sterile Petri plates, dispersed equally throughout the plates, and filled with sterile unsolidified nutrient agar, which was then let to solidify. The plates of nutrient agar were incubated. For 24 hours at 37°C. The bacterial colonies were separated and purified from the plates following incubation. The healthy bacterial cultures are kept at 4°C and used for additional screening methods.1

Screening

The capacity of fifteen bacterial isolates with different morphologies to break down the textile dyes was examined. By culturing the isolated bacterial strains on 100 milliliters of nutritional agar medium with 10 milliliters of dye wastewater, they were filtered out. For twenty-four hours, the nutrient agar medium was incubated at 37°C. Plates were checked for a clean zone following incubation. For additional research, the screened culture was moved to an agar slant and stored at 4°C. Five bacterial isolates with different morphologies demonstrated over 70% degradation of the added dye effluents. These effective types of bacteria were chosen for additional research.

Dye decolorization experiments

Dye decolorization experiments were carried out in three 250 ml Erlenmeyer flasks for three effluent samples. Each flask contains 100 ml of Nutrient Broth with 15 ml of dye effluents. The pH was adjusted to 7± 0.2. Then, the flasks were autoclaved at 121°C at 15 lbs pressure for 15 minutes. The autoclaved flasks were inoculated with 5 ml of bacterial inoculumsfor each isolate and bacterial consortium. The flasks were kept in a mechanical shaker and incubated at 37 ºC for 4 days. Samples were drawn at 24-hour intervals for observation. About 10 ml of the dye solution was filtered and centrifuged at 5000 rpm for 20 minutes. Decolorization was assessed by measuring absorbance at 510 nm of the supernatant with the help of a spectrophotometer at wavelength maxima (λm) of the respective dye [13].

Optimization of dye concentration

The maximum decolorization of the three different azodyes by the selected strain was tested at different time interval (0hrs, 24hrs, 48hrs, 72hr and 96hrs) in broth medium, and the percent of dye degradation was calculated as previously mentioned [14, 15].

RESULTS

Sample collection

Effluent samples were successfully collected from the dyeing units located in Tirupur and Coimbatore regions. A total of five samples were obtained and labeled as S1 to S5 based on their collection locations. The physical appearance of the samples varied slightly in color and turbidity, indicating differences in dye composition and concentration among the sources. The plastic cans used for collection, pre-washed with tap and distilled water, ensured minimal contamination and preserved the integrity of the samples for subsequent analysis (9). Initial visual observation revealed that all samples had a deep coloration ranging from reddish-brown to dark violet, characteristic of synthetic dye effluents discharged from textile industries. The uniformity in the sample labeling enabled systematic tracking and comparative analysis in downstream procedures.

Isolation of the dye-degrading bacterial isolates from dye effluent

Bacterial isolates were successfully obtained from the textile dye effluent samples using the serial dilution pour plate method. A clear gradient in colony density was observed across the dilution series, with countable and well-isolated colonies appearing predominantly at 10^{-4} to 10^{-6} dilution levels. After 24 hours of incubation at 37°C, distinct bacterial colonies with varying morphologies (in terms of shape, size, color, and texture) were observed on the nutrient agar plates. These colonies were carefully subculture to obtain pure isolates. The purified and healthy cultures were maintained at 4°C on nutrient agar slants for further screening and dye decolorization studies.

Fig.1. Isolation and Biochemical test for Isolate B1

Table.1. Biochemical test for Isolate B1

S. No	Tests	B1 *Bacillus cereus*
1	Gram reaction	Gram-positive
2	Motility	Motile
3	Shape	Straight rod
4	Spore	Positive
5	Catalase	Positive
6	Oxidase	Negative
7	Indole production	Negative
8	Methyl red	Negative
9	Voges-Prosakauer	Positive
10	Citrate	Positive
11	Urease	Negative
12	Starch hydrolysis	Positive
13	Nitrate reduction	Positive
14	Glucose utilization	Positive
15	Lactose utilization	Negative
16	Gelatin	Negative
17	H_2S	Positive
18	TSI	Positive

Screening

Screening experiments were performed for different bacterial isolates. Among the four different azo dye degrading bacterial isolates the most efficient isolates such as B1were used in degradation studies. B1

strains grew well on medium containing Mordant black. Thus among the strains of *Bacillus cereus* examined and based on decolorization efficiency strains B1 appeared to be the versatile organisms for effective decolorization. Biochemical characteristics were depicted in Table 1; Fig. 1, Based on these tests the isolates B1 were confirmed as *Bacillus cereus* and they were further examined for their dye decolorizing ability. Among the isolates, B1 decolorized the dyes effectively. The strain B1 revealed positive reactions for, catalase, oxidase, citrate, nitrate reduction, glucose utilization, and gelatin liquefaction test.

Fig. 2. Dye decolorization experiments

Table 2: Time dependent reduction in absorbance (OD) of dye during bacterial biodegradation.

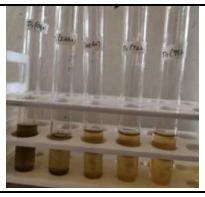
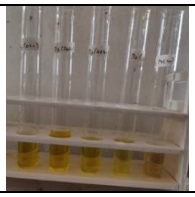
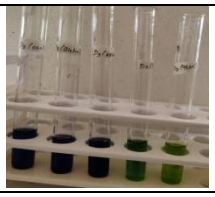
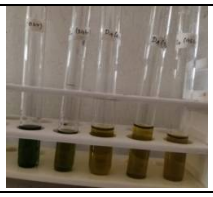
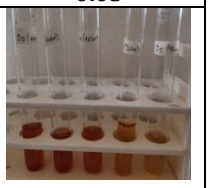
No. of. Hours	Name of the dye				
	D1	**D2**	**D3**	**D4**	**D5**
0hr	**0.93**	**0.90**	**1.20**	**1.01**	**1.10**
24hrs	**0.82**	**0.70**	**0.95**	**0.93**	**0.80**
48hrs	**0.63**	**0.42**	**0.63**	**0.70**	**0.50**
72hrs	**0.42**	**0.18**	**0.32**	**0.63**	**0.22**
96hrs	**0.30**	**0.04**	**0.10**	**0.10**	**0.05**
Image					

Table 3: Time-dependent decolorization (%) of synthetic dyes by bacterial isolate

Name of the dye	Percentage of degradation:
D1	**67.7%**
D2	**95.5%**
D3	**91.6%**
D4	**90.09%**
D5	**95.4%**

Optimization of dye concentration

Fig. 2; Table 2 depicted the change in the extent of dye decolorization in response to varying incubation condition, pH, temperature, dye concentration and inoculums size. The best decolorization exhibited with static incubation at pH 7**.** The results showed that the decolorization rate increased with increment of temperature from 25-37. With an increment of inoculums size, the extent of decolorization also increased and 97.4 % decolorization was achieved in 10 % (v/v) of inoculums. A further increase in volume of inoculums resulted in decreased decolorization efficiency of the bacteria**.** Similar studies have been reported [16, 17]. where a decrease in the efficiency of decolorization was observed with increase in initial dye concentration. With subsequent increase in dye concentration toxic effect of dye and its metabolites became dominant, leading to inhibition in decolorization [18].

The decrease in the efficiency of color removal with increase in concentration of dye can be due to toxic effect of dye and inadequate amount of biomass to uptake this higher concentration of dye and the ability of the enzyme to recognize the substrate efficiently at the very low concentrations [19, 20]. With the increase of inoculums size gradually decolorization efficiency also increased up to certain limit. Further increasing causes decreased decolorization. [21] Reported the similar result early depletion of nutrient occurs as the inoculums volume increased and hence biological process of decolorization involving microorganisms require an optimum number of microbial cells. There was no change in the colour decolorization within 08 h, possibly indicating the lag phase for adaptation to the environment. After 24 h, decolourization was visualized. Novacron black and Novacron blue dk dye take additional time

compared to other dyes. This phenomenon occurs owing to the complex structure and nature of textile dyes [22, 23]. Decolourization of Azo dye by all five single isolates and consortium was studied and visualized.

Molecular Characterization and Phylogenetic Analyses

The isolate (B1) was identified by BLASTn results, and taxonomy designations were given based on the NCBI database nearest neighbour. The bacterial strains' BLASTn analysis is shown in Table 4. The isolate B1 (PV875250), have accession numbers. Fig. 3 and 4 displays the phylogenetic connection of the isolate, with an ideal branch length total of 2.013. Both trees are depicted as legends in the illustration and are drawn to scale.

Evolutionary relationships of isolate, 2 of their nearest neighbors and 6 taxa

The evolutionary history was inferred using the Neighbor-Joining method showed in Fig. 4; Table 4[24] the optimal tree with the sum of branch length = 2.013 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches [25]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method with Standard error and are in the units of the number of base substitutions per site. The analytical procedure encompassed 9 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 1,591 positions. Evolutionary analyses were conducted in MEGA12 [26, 27] utilizing up to 7 parallel computing threads in Table 2.

Evolutionary analysis by the Maximum Likelihood method

The phylogeny was inferred using the Maximum Likelihood method and [28] 2-parameter model [29] of nucleotide substitutions and the tree with the highest log likelihood (-4,477.04) is shown. The percentage of replicate trees in which the associated taxa clustered together (1,000 replicates) is shown next to the branches [30, 31]. The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the 2-parametermodel. The MP tree had the shortest length among 10 MP tree searches; each performed with a randomly generated starting tree. The analytical procedure encompassed 9 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions [33]. The partial deletion option was applied to eliminate all positions with less than 95% site coverage resulting in a final data set comprising 815 positions. Evolutionary analyses were conducted in MEGA12 [34]. utilizing up to 7 parallel computing threads [32].

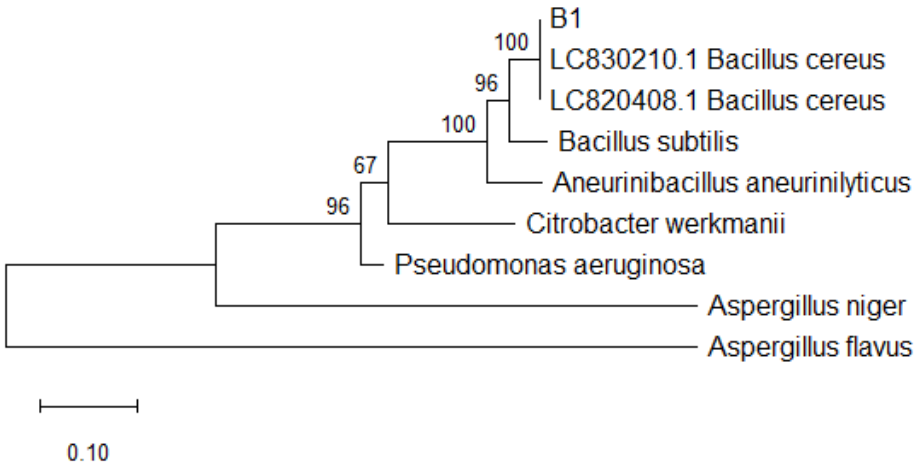


Fig.3. Neighbor-Joining tree showing the phylogenetic relationship amongst the strains. The scale bar represents 0.10 nucleotide substitutions per sequence position.

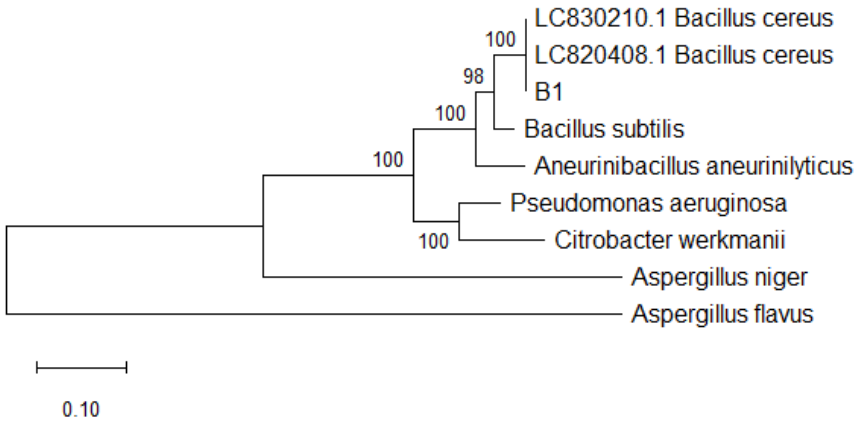


Fig.4. Maximum Likelihood tree showing the phylogenetic relationship amongst the strains. The scale bar represents 0.10 nucleotide substitutions per sequence position.

Table.4. Estimates of Evolutionary Divergence between Sequences

	B1	***LC830210.1 Bacillus_cereus***	***LC820408.1Bacillus cereus***	***Pseudomonas_aeruginosa***	***Aneurinibacillus_aneurinilyticus***	***Aspergillus_niger***	***Citrobacter_werkmanii***	***Bacillus subtilis***	***Aspergillus_flavus***
B1		0.00	0.00	0.02	0.01	0.04	0.02	0.01	0.10
LC830210.1_Bacillus_cereus	0.00		0.00	0.02	0.01	0.04	0.02	0.01	0.10
LC820408.1_Bacillus_cereus	0.00	0.00		0.02	0.01	0.04	0.02	0.01	0.10
Pseudomonas_aeruginosa	0.22	0.22	0.21		0.02	0.04	0.01	0.02	0.08
Aneurinibacillus_aneurinilyticus	0.12	0.12	0.12	0.22		0.04	0.02	0.01	0.10
Aspergillus_niger	0.82	0.82	0.82	0.65	0.80		0.05	0.05	0.13
Citrobacter_werkmanii	0.29	0.29	0.29	0.16	0.28	0.88		0.02	0.09
Bacillus_subtilis	0.07	0.07	0.07	0.22	0.11	0.85	0.31		0.10
Aspergillus_flavus	1.25	1.25	1.24	1.12	1.30	1.42	1.22	1.28	

Table. 5. The assigned taxonomic name of the bacterial strain B1, along with the GenBank accession number of the nearest neighbor

Isolate	**Name**	**GenBank Accession Number of nearest neighbors**	**Identity %**
B1	*Bacillus cereus*	LC830210	99.80%
	Bacillus cereus	LC820408	99.73%
	Bacillus cereus	KF114432	99.12%
	Bacillus cereus	KU902033	99.05%

The numbers of base substitutions per site from between sequences are shown. Standard error estimate are shown above the diagonal [35]. Analyses were conducted using the Kimura 2-parameter model. The analytical procedure encompassed 9 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 1,591 positions. Evolutionary analyses were conducted in MEGA12 [36, 37].

CONCLUSION

The present study demonstrates the remarkable ability of a single bacterial isolate to degrade a wide range of textile dyes, including Methylene Blue, Crystal Violet, Congo Red, Malachite Green, and Reactive Black. The bacterium showed efficient decolorization across different dye classes, indicating its broad-spectrum enzymatic activity and adaptability to various dye structures. These findings suggest that this bacterial strain holds significant potential for application in the bioremediation of dye-contaminated wastewater. Further optimization and scale-up studies could pave the way for its effective use in industrial effluent treatment systems.

DECLARATIONS OF COMPETING INTEREST

The authors declared that there is no conflict of interest

DATA AVAILABILITY

The data sets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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ETHICAL APPROVAL: Not applicable.

CONSENT TO PARTICIPATE: Not applicable.

CONSENT FOR PUBLICATION: All the authors agreed to publish the data in this journal.

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