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Isolation, Identification, and Characterization of Putative Dye-Degrading Bacteria from Polluted Soil: Bioremediation Investigations

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ABSTRACT

The residual dye within the soil from the synthetic dye manufacturing and fabric industries is a global state of affairs. The discharge consists of an excessive content of pigments and other components, creating complicated structures. It leads to damage to the soil structure and its fertility. Amid existing amputation methods, microbial remediation takes significant consideration owing to its subordinate charge, sophisticated proficiency, and fewer influences on the milieu. The current study was premeditated for the seclusion and portrayal of azo dye- dye-decolorizing bacteria, which is a criterion for emerging a microorganism-facilitated treatment of adulterating dyes. In this present investigation, twenty sorts of bacteria that were talented to decolorize seven kinds of azo dyes (Crystal Violet, Methylene Blue, Safranine, Congo Red, Methyl Orange, Malachite Green, and Carbol Fuchsin) were isolated from dye-polluted soil from the dying industry near the railway station; in Calicut. Based on 16S rDNA scrutiny, the most resourceful decolourizing bacteria for these azo dyes was identified as *Priestia megaterium* strain *NRBC 15308*. After characterization, *Priestia megaterium* was found to be optimally nurtured at 35°C, on a pH of 7, with a 1.5% glucose concentration in a minimal salt medium. 100% decolorization of a 6% dye solution was found at optimal conditions by *Priestia megaterium*. *Priestia megaterium* can decolorize cotton and gauze suspended in the dye solution in 24 hours. Bioremediation studies with the isolate proved that the inhibition effect of the dye solution on seed germination could be removed by the application of *Prestia megaterium*. The isolation of *Priestia megaterium* strain NRBC 15308 as a dye-degrading bacterium holds immense promise for remediating dye-contaminated soil.

INTRODUCTION

Soil performs a vital role in the environment. complex composition of inorganic factors (minerals) derived from disintegrated rocks, humus, water, and so on. Conjointly have an impact on the homes of the soil. The same old American Society for Checking Out and Substances (ASTM) model of the Unified Soil category device has categorized soil on the premise of grain size and texture in clay, gravel, organic clay, natural slit, peat, sand, and silt (ASTM 2017). The clay fraction has an excessive floor area and holds many chemical and biological properties of arable soil. While sand and silt fractions ordinarily manage the bodily person, In contrast to minerals, the soil consists especially of number one (chemically unaltered) and secondary (chemically altered) rocks, silicates and non-silicates, and crystalline and non-crystalline minerals (Schulze 2002). Likewise, Kumar et al. (2024) argue biotechnological advancements are essentially dangerous to the environment because they have the potential to reduce metal pollution. Pollutants in the environment can be efficiently removed using bioremediation. Both native and introduced species can thrive in a microorganism-friendly environment.

Alongside this, soils provide anchorage to roots, clench water, and provide nutrients. As cliché goes, "We build on the soil as well as with it and in it." The attention to heterogeneous materials varies due to many factors, which include metals, chemical compounds (monomers, solvents, reactive initiators, dyes, and pigments) leaching from municipal and chemical industries, unintentional spillage, and underground leakage that cause soil pollutants (Calacea et al. 2005). Those troubles are of primary concern as the contaminated websites are considerably expanding and growing to be appealing. Inside America, more than 40,000 websites have been determined to be contaminated with the above contaminants, in accordance with a document by the Environmental Safety Corporation (EPA) (Sharma & Pandey 2014, Zhao et al. 2016).

The removal of contaminants from soil depends on the complicated interaction of physical, chemical, and organic factors within the surroundings. The bioremediation era is a powerful approach that utilizes organisms to neutralize pollution (Baocheng et al. 2008). The bioremediation procedure of dye remedy from infected soil could be very powerful and can be called a sustainable method. It's a low-budget and effective treatment manner. The consumption of the strength all through the remedy of dye-containing soil is very low. Remedy of carcinogenic dye may also be very beneficial by way of the usage of microbes, as microbes digested the level of carcinogenicity of the dye. Yadav et al. (2021) confirmed that laccase is responsible for lessening the level of dye carcinogenicity. The authors used *Allium cepa* to check the toxicity and observed that laccase reduced the toxicity of the dye.

Microbial degradation of hydrophobic compounds takes place because of their shape, the availability of microorganisms, and the physico-chemical situations that affect the metabolic competence of the microorganisms (Heng et al. 2008). It consists of various good-sized parameters, which include pH, water, aeration repute (redox ability), presence of oxygen, vitamins, and temperature. Biodegradation of organic contaminants can be performed as it should be, but in keeping with some authors, microorganisms and their ecology need to be fully understood. Holden et al. (1997) cautioned against deciding the elements regulating the preferred metabolic pathway and the distribution of degrading microbial groups relative to pollutants.

A lot of bodily and chemical treatment techniques, together with oxidation, reduction, adsorption, chemical precipitation and flocculation, electrochemical remedy, and ion-pair extraction, are used to do away with dyes from wastewater effluents (Fan et al. 2009). Those strategies are attractive due to their performance; however, they are complex and steeply priced (Robinson et al. 2001). On the other hand, biological techniques have obtained revolutionary attention because of their fee, effectiveness, capability to produce much less sludge, and environmental harmlessness. Those procedures can convert this complex natural pollution into water, carbon dioxide, and inorganic salts (Daneshvar et al. 2007). A huge variety of microorganisms can decolorize or maybe completely mineralize an extensive variety of dyes, along with microorganisms (Dave & Dave 2008), fungi, and algae (Ghanem et al. 2011, Ramya et al. 2010). The most promising microorganisms for wastewater remedy are the ones remoted from websites infected with dyes (Indigenous) due to the fact they've adapted to survive in negative situations (Dave & Dave 2008).

A study carried out with the sample dye-infected water showed ninety-five% degradation of all dyes by *Bacillus megaterium*, and then they concluded that *Bacillus megaterium* can be used for the treatment of the dye-infected soil and water (Lekha & Nair 2017).

Based totally on laboratory effects, efforts have to then be made to scale up and apply bacterial decolorization strategies in real industrial effluents. In addition, with the trendy advances in genomics and proteomics, there's a possibility to improve the overall performance of bacterial or enzymatic remedies for textile wastewater. With all the advantageous study findings and ongoing tendencies, microbiological treatment is hoped to be essential in the elimination of dyes and poisonous chemical compounds in fabric wastewater. In addition, the sample from textile wastewater effluent was determined to contain *Bacillus megaterium* KY848339.1, a bacterial stress that should remove ninety-one percent of AR377 azo dye at the concentration of 500 $mg.L^{-1}$ within 24 h (Tahir et al. 2021). From estuarine sediments (Velar Estuary, Porto novo, Tamil Nadu, India), isolate *Bacillus megaterium* PNS15 confirmed an appreciable synthesis of dye-degrading enzymes (azo reductase and laccase). Sivasubramani et al. (2021) selected the isolate that changed into *Bacillus megaterium* and confirmed maximum decolorization of turquoise blue dye within forty-eight hours at pH 7.00. The isolated subculture can decolorize turquoise blue dye awareness by as much as 5 mg in herbal conditions. The remote pressure is even able to degrade a huge variety of dyes. Similarly, *Bacillus megaterium* species may be carried out and examined at large-scale degradation of this dye (Joshi et al 2013). The present investigation aims to isolate and identify an indigenous bacterial strain from dye enterprise effluent that can degrade diverse azo dyes. Compare and optimize the biodegradation functionality of such traces and their application in bioremediation.

MATERIALS AND METHODS

Collection of Soil Samples

The sample was collected from soil contaminated with dyes from a dying industry near a railway station in Calicut, Kerala (Fig. 1). The soil sample is highly polluted with dye and other chemicals used in the dying industry. The soil sample had an alkali pH of about 9.5. The soil was dark in color. The soil was air-dried to remove excess moisture, and the samples were minced into a fine powder via a grout and pounder and used for study.

Preparation of an Azo dye Solution

1% standard solutions are prepared by dissolving 1 g of a piece of powder dye into 100 mL of autoclaved purified water, followed by percolation. Table 1 shows the composition of azo dye.

Selective Media Used for the Study

Minimal salt agar: Prepare MS medium supplemented with a 1% azo dye solution for primary screening studies.

Preliminary screening: The screening methods were based on the modified methodology of Sriram et al. (2013). The minimal salt agar prepared was inoculated with a serially diluted soil sample with a 1% dye solution for preliminary screening. The colonies developed on the plates were subcultures and used for further screening.

Fig. 1: Soil sample used for the study.

Table. 1: Composition of Azo Dye.

Crystal Violet	1 mL
Methylene Blue	1 mL
Safranine	1 mL
Congo Red	1 mL
Methyl Orange	1 mL
Malachite Green	1 mL
Carbol fuchsin	1 mL

Secondary screening: For each subsequent acclimatization step, MSA plates were prepared with incremental increases in dye concentration (e.g., 2%, 3%, 4%, 5%, and 6%), and the colonies isolated in the initial screening were inoculated into these plates and incubated at 37°C. The organism accomplished the highest concentration of dye solution, which was used for further studies (Sriram et al. 2013)

Identification of Selected Bacterial Strains (Afrin et al. 2021) (Bergey's Manual)

The isolated bacterial culture was enhanced in the nutrient broth medium. Later, the 24-hour streaking plate method was done through nutrient agar media; therefore, sole and untainted colonies were electrified, which meant forced entails. These microbial strains were identified through morphologic and gram-staining physical appearance, biochemical, and molecular credentials classification**.**

Morphological and Biochemical Classification

The quarantines were identified based on morphological studies and biochemical characterization. Bergey's manual was rummage-sale as a reference to identify the isolates. Microscopic features were recorded for all colonies: color, size, and shape, and then further staining was done. Isolates were analyzed for motility test by hanging drop technique, oxidase, catalase, indole, MR-VP, citrate utilization, and Urease test (Garrity et al. 2006)

Molecular Characterization

The complete identification of the isolate was done according to the method suggested by Saitou and Nei (1987)**.** The genome of the isolate was isolated, amplified, and analyzed.

Isolation of Genomic DNA from an Isolated Culture and PCR Amplification

Isolation of DNA was done by using the Hi Pur ATM Bacterial Genomic DNA Purification Kit (MB505, Himedia). The polymerase chain reaction cast off to the 16S rDNA sequencing with forward primer: 5'GAGTTTGATCCTGGCTCA 3' and reverse primer: 5'ACGGCTAACTTGTTACGACT 3'were purchased from Eurofins, Bangalore. The PCR was performed with the following modification: the reactions were run with 10 µL of Master Mix (Emerald Amp GT PCR, 2X premix), 0.2µ M forward and reverse primers, DNA <500ng, and molecular biology grade water. We performed the amplification via the ensuing cycling conditions: 98°C for 30 s, followed by 30 cycles of 98°C for 10 s and 55-60°C for 30 seconds, then 72°C for 1 min, and a final extension at 72°C for 10 min The amplified PCR products were visually proven under ultraviolet light via a 1% agarose gel stained

with ethidium bromide (EtBr). The amplified products were succumbed for sequencing through both forward and reverse primers at Macrogen, Inc.

Phylogenetic Tree Construction

The PCR product is then subjected to a cleanup procedure, followed by sequencing using 27F and 1492R worldwide primers. Sequencing reactions were executed with an ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kit with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). The sequencing file remained open in Snap Gene Viewer, and the raw data was checked for the length of the quality and read. The sequence is then blasted using the BLASTn tool to identify the sequence of the organism to which the data is aligned with maximum score and coverage. A phylogenetic tree was constructed using MEGA 11.0.13 version software with the neighbor-joining hood method.

Dye Decolorization Assay

Minimal salt broth medium amended with 2% dye solution was inoculated with microbial quarantine (1% v/v) and incubated at 37°C under a shuddering condition. Samples were withdrawn after 24 h and analyzed for growth and decolorization. Decolorization was determined by measuring the absorption spectra at the lambda max (λ max) of the dye (410 nm) for the clear supernatant and growth using the absorbance of the culture supernatant (640 nm) using a spectrophotometer. A medium containing dye without the inoculum was considered a control. The color elimination efficacy was specified as the percentage ratio built on the ensuing equation. Respectively, testing was executed in triplicate, and the mean values were documented.

Percent decolorization = (initial absorbance – Final absorbance value) ×100/Initial absorbance

Optimization Studies

All the activities of the organisms will be at their maximum in their optimal conditions. So, the optimization of environmental and nutrient conditions like media composition, pH, temperature, and concentration of glucose as a co-substrate was conducted to ensure the maximum enhancement of the decolorization property of the organism.

Optimization of Media

Minimal Salt Broth, Minimal Mineral Broth, and Bushnell Haas Broth were selected for the study, and 50 mL of each medium was inoculated in one ml culture of the quarantine and incubated for 24 hours at 37°C. Media that showed maximum color removal efficiency was used for further degradation studies. Individual trials were executed in triplicate, and the mean values were verified.

Optimization of pH, Temperature Glucose, and Salinity Levels

Optimization of various parameters like pH (3.5, 4.5, 7, 8.5, 9.5.), temperature (15°C, 25°C, 35°C, 45°C, 55°C), glucose (0, 1%,1.5%, 2%, 2.5%), and salinity levels (1, 3, 5, and 7 % NaCl) for decolorization of azo dye solution was complete with some amendments by Shah (2013) and Prasad and Rao (2013). The enhanced media with 5% (v/v) dye solution was inoculated with a 1% (v/v) microbial isolate and incubated for 24 hours. Uninoculated medium with azo dye solutions was kept back as a control. Respectively, research was accomplished in triplicate, and the mean standards were documented.

Application Studies

The isolated bacterium, after optimization and decolorization assay, was used to analyze the efficiency of color removal from cotton and gauze and also to understand the bioremediation capacity of the organism.

Cotton and Gauze Roll Decolorization Study

Minimal salt broth amended with 5% (v/v) of azo dye solution was inoculated with 1 ml of the isolated bacterium. Cotton roll and Gauze roll, each weighing 2.340 g, were added to the medium in separate flasks and incubated under optimized conditions for 24 h. A control was maintained without adding any organisms to the medium. The color removal efficiency of the organism from the test materials was calculated using the formula along with the growth of the organism in the medium as described above.

Bioremediation Studies (Ajisha et al. 2021)

Seeds used: Okra seeds were collected from the "green leaves" nursery in Vellangallur, Thrissur. For this experiment, we used okra seed due to its fast germination. The procedure was done in aseptic conditions.

Soil used: A red loamy type of soil, which was collected from the SIAS campus in Vazhayur East, was sterilized for three consecutive days to avoid any spore and fungal contamination. The soil pH of 6.1 also ought to have a virtuous aquatic holding capacity.

Germination test in soil: The bushy cell deferment of *Priestia megaterium* cells prepared stood castoff for inoculating the loam. 3 ml of the cell suspension was further added to 60 gm of dirt containing 20% moisture and 6% and 8% dye, engaged in flexible cups, and assorted meticulously to guarantee even dispersal. Seeds were sown immediately,

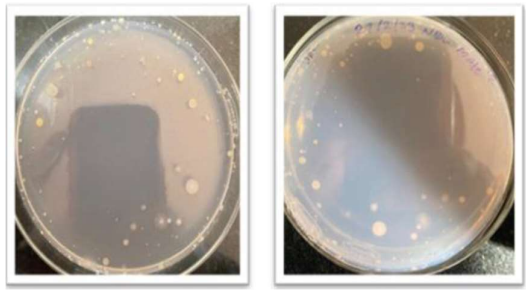

Fig. 2: Primary screening.

separately in five sets with triplicates: set 1 (soil+ seed), set 2 (soil+ seed+ organism), set 3 (soil+ seed+ dye), set 4 (soil+ seed+ dye (6%) + organism), and set 5 (soil+ seed+ dye (6%) + organism).

RESULTS AND DISCUSSION

Screening and Isolation of Azo Dye-Debasing Microorganisms

The serially diluted sample plated on the minimal salt agar with 1% of the dye solution produced different types of colonies after incubation for 24 h. The bacterial colonies were marked as D01 to D20, and the fungal colonies found on the plates were named F01 and F02. The detailed colony morphology of the isolates is given in Table 2. The organisms that could utilize the dyes provided in the medium as a carbon source showed growth in the medium (Fig. 2).

Secondary Screening

When the MS agar plates had an incremental increase in dye concentrations of 2%, 3%, 4%, 5%, and 6%, only one bacterium named D20 could survive. Mahmood et al. (2012) and Karthikeyan & Anbusaravanan (2013) have stated the seclusion and screening of microbes were accomplished by decolorizing several azo dyes since sludge samples collected from sewer water handling places polluted with dyes in the comparable method. Jamee & Siddique (2019) observed that synthetic dye degradation by microbes is facilitated by their oxidase. Prevalently, the azo reductase places the utmost dynamic part in decolorization by breaking down azo bonds. Microbial oxidoreductase enzymes are significant in the squalor of artificial dyes. This energetic metabolism of microorganisms empowers them to exploit multifaceted xenobiotic amalgams of the dyestuff as a substratum. Among the development, they are cracked down to a reduced number

Table 2: The colony morphology of various organisms grown in the MS agar on preliminary screening.

Organism	Shape	Color	Margin	Opacity	Dry/wet	Size
D1	Circular	Yellow	Regular	Opaque, flat	Wet	Medium
D2	Circular	Pinkish white	Irregular	Opaque, flat	Dry	Small
D3	Circular	Light yellow	Regular	Opaque, mucoid	Dry	Small
D4	Circular	Off white	Irregular	Opaque	Wet	Small
D5	Circular	Cream	Regular	Opaque	Wet	Medium
D6	Circular	Light white	Regular	Opaque	Wet	Medium
D7	No shape	Pinkish white	Irregular	Opaque	Dry	Small
D8	Circular	Yellow	Regular	Opaque	Wet	Large
D9	Circular	Yellow	Regular	Opaque	Dry	Medium
D10	Circular	Yellow	Regular	Opaque	Wet	Small
D11	Circular	Pinkish white	Regular	Opaque	Wet	Small
D12	Circular	White	Regular	Opaque	Wet	Large
D13	Circular	Pinkish white	Regular	Opaque	Wet	Small
D14	Circular	White	Regular	Opaque	Dry	Medium
D15	Circular	Transparent	Regular	Transparent	Wet	Medium
D16	Circular	White	Irregular	Opaque	Dry	Small
D17	Circular	White	Regular	Opaque	Wet	Small
D18	Circular	White	Regular	Opaque	Dry	Medium
D19	Circular	Pink	Irregular	Opaque	Wet	Medium
D20	No shape	Milky white,	Irregular	Opaque	Wet	Large
F01	A	White	Fungal	Colony	-	-
F02	A	Black	Fungal	Colony	-	-

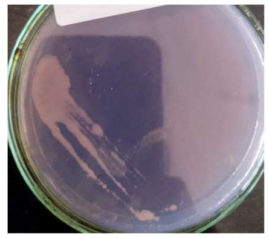

Fig. 3: Secondary screening.

of intricate metabolites. A benefit of procuring microbes from authentic places of effluent removal is that they are more likely to partake in the enzymes stimulated, which enable the putrefaction of dyes. Fig. 3.

Identification of the Selected Bacterial Strain

The morphological and biochemical characterization of isolated bacteria was studied. Findings are tabulated in Table 3 and Fig. 4.

Molecular Identification

D20 genomic DNA was isolated and verified (Fig. 5); the template (DNA) was used to amplify the 16SrRNA. DNA sequence by PCR (Fig. 6) The PCR product was then subjected to PCR clean-up followed by sequencing. The nucleotide sequence obtained after sequencing was blasted using the BLASTn program. The bacteria were identified as *Priestia megaterium NRBC* 15308=*ATCC* 14581. Sanger sequencing, also known as the Hawser termination technique, is used to determine the nucleotide sequence of DNA. Coli DNA polymerase l (pol l) or its proteolytic (Klenow) fragment was chosen by Dr. Sanger for his dideoxy sequencing chemistry (Sanger et al. 1977). The rudimentary local alignment search tool (BLAST) finds regions of local resemblance among sequences. The program likens nucleotide or protein sequences to sequence databases and computes the statistical implications.

Table. 3: Biochemical characteristics (Positive+, Negative-).

S. No.	Characters	Result
1.	Gram staining	Gram positive diplobacillus
2.	Morphology	Milky white, irregular, mucoid, raised
3.	Motility	motile
4.	Spore	Spore forming
5.	Indole	Negative
6.	Methyl Red	Negative
7.	Voges-Proskauer	Positive
8.	Citrate test	Positive
9.	Catalase test	Positive
10.	Oxidase test	Negative
11.	Urease	Positive

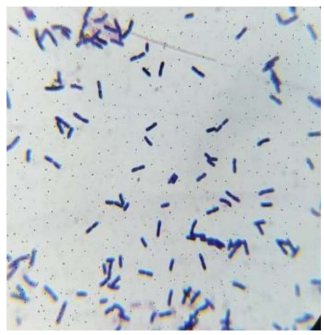

Fig. 4: Gram-positive diplobacillus.

Phylogenetic Tree

Phylogenetic investigation delivers an in-depth understanding of how species progress through genetic vicissitudes (Fig. 7). Through phylogenetics, experts can appraise the pathway that links a current organism with inherited derivation, as well as forecast the heritable discrepancy that might arise in the future. Subsequently, in molecular identification, the isolate was identified as *Priestia megaterium* (NRBC 15308=ATCC 14581, Fig.7). Have 1.5 kb of molecular weight, wi*th* 99% identities. Maximum score: 1376, E-value: 0.0, accession number: NR1126361. The research was done by Afrin et al. (2021) and found that

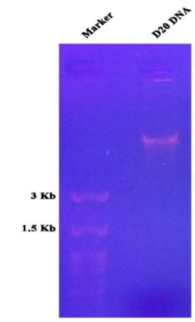

Fig. 5: DNA isolated from D20 run in 0.8% agarose gel.

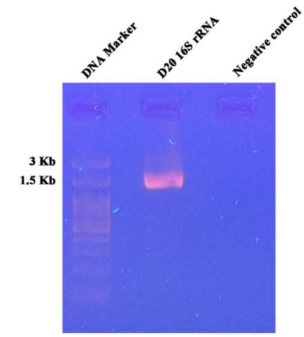

Fig. 6: PCR amplification of 16S rRNA of D20.

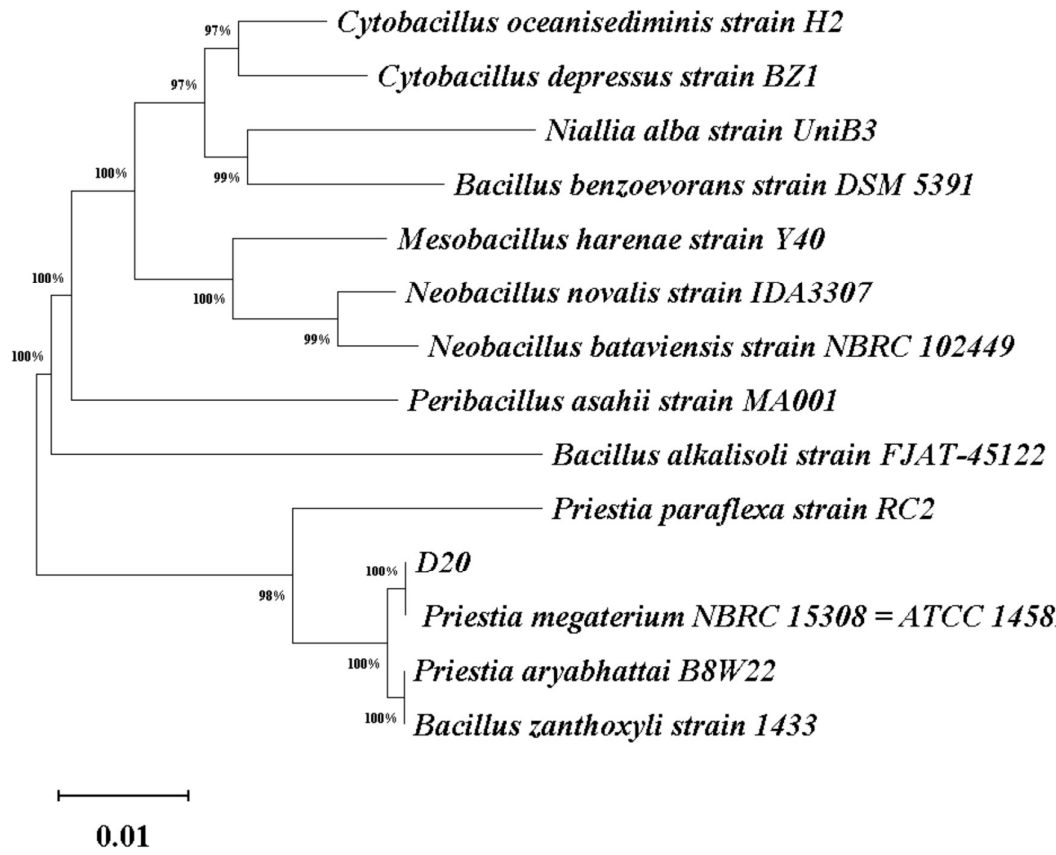

Fig. 7: Phylogenetic placement of 16S rRNA sequences.

similar organisms can degrade maximum dye: *Enterococcus faecium* and two strains of *Pseudomonas aeruginosa* after molecular identification. The maximum decolorization occurred in the range of 37ºC under aerobic conditions. Similarly, the research done by Sulthana (2017) identified three important bacterial species, *Enterococcus faecium, Bacillus pumilis,* and *Bacillus thuringiensis,* through a biological identification system. A soil sample was collected from local textile effluent. Another research (Fareed et al. 2022) found that the maximum decolorization by the bacteria was identified as *Bacillus cereus* strain *ROC*. The soil sample was collected from contaminated industrial areas. The research was done by Fareed et al. (2022), who identified *Bacillus cereus ROC* as being highly proficient in degrading dyes with a decolorization rate of 83%.

Dye Decolorization Assay

The test done in triplicate showed an average of 82.4 % degradation when provided with 2% dye Table 4.

Optimization of Media

Media optimization studies with different media like Minimal Salt Broth, Minimal Mineral Broth, and Bushnell Haas Broth proved that Minimal salt agar is the most efficient media for maximum decolorization of dye. One flask from each is considered a control. Inoculated 100 µl of inoculum into 3 broths. I observed the color change after overnight incubation. The decolorization of dye by *Priestia megaterium* in the tested media is provided in Fig. 8. Observed the maximum decolorization efficiency (100%) in minimal salt broth compared to minimal mineral broth and Bushnell Haas broth Table 5. The organism could complete decolorization of 5% of the azo dye solution; normally, P*riestia megaterium* is grown in minimal media. Radia Jamee & Romana Siddique

Table 4: Decolourisation rate of Decolourisation rate of 2% of azo dye in the minimal salt medium at OD 410nm.

Conditions	Control	MS Broth (1)	MS Broth (2)	MS Broth(3)
Before inoculation	0.00	0.00	0.00	
Initial absorbance	0.00	0.17	0.16	0.17
Final absorbance	0.00	0.03	0.02	0.03
Percentage of decolorisation	0	82.4	87.5	82.4

Table 5: Optimisation of media OD at 410 nm.

Conditions	Control	Minimal Salt Broth	Minimal Mineral Broth	Bushnell Haas Broth
Before inoculation	0.00	0.00	0.00	0.00
Initial absorbance	0.00	1.27	1.22	1.25
Final absorbance	0.00	0.00	0.17	0.12
% of decolorisation	0	100	86	90.4

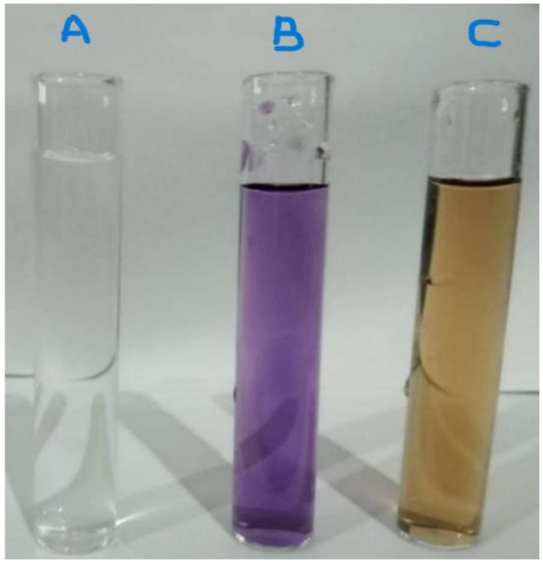

Fig. 8: Supernatant of the decolorized medium after 24 hours A- Minimal Salt Broth, B- Bushnell Haas Broth, C- Minimal Mineral Broth.

(2019) observed that the filth of artificial dyes by microbes is eased by their oxidases. In the instance of azo dyes, azo reductase plays the most vigorous part in decolorization by flouting down azo bonds. Approximately microbes have been deliberately to worsen dyes below the aerophilic state. The current study shows its maximum decolorization was 100% (Table 5).

Optimization of pH, Temperature and Glucose

The selected D20 organism has optimal growth conditions like pH, temperature, and glucose. Here, the decolorization amount is higher at the ideal pH and decreases with further acidic or alkaline pH; the optimal pH is between 7 and 8.5. Besides, it remains experiential that a cumulative temperature of 35°C - 45°C raises the decolorization percentage; further cumulative temperature might decrease the decolorization rate.

Optimization of pH

The research revealed that the ratio of azo dye degradation had improved with the modification of pH in the broth. The higher dilapidation remained pragmatic at pH 7. The optimal decolorization was at pH 7 (100%), even though the organism could decolorize the dye at an alkaline pH of 9.5 (92%). But at an acidic pH, the organism gets less reactive. The azo dye had an alkaline pH so that *Priestia megaterium* could degrade the azo dyes at this pH level. Several researchers have evidenced that biosorption developments via microorganisms remained highly pH-dependent (Aksu & Tezer 2005, Kumar et al. 2006). According to popular alternative research done by Wang et al. (2009), the *Citrobacter* sp. CK3 must have accomplished the finest decolorization of the sensitive red 180 (96%) at pH 6.0-7.0. According to Bishnoi et al. (2024), phenanthrene degradation increased with an increase in pH from 5.0 to 7.0 and again decreased with a further increase in pH from 7.0 to 9.0. Maximum degradation of PAH was observed at pH. In the instance of red azo dye decolorization by *Aspergillus niger*, it might be detected that the exclusion percent upsurge with the rise of pH and the extreme decolorization competence stood touched (99.69) at pH 9.0 (Mahmoud et al. 2017). In my study, the azo dye solution was completely decolorized (100%) by *Priestia megaterium* at pH 7 (Table 6).

Optimization of temperature

The maximum (100%) of azo dye decolorization was observed at 35ºC as represented in Table. 7: Microbes need an optimal temperature for development. Meanwhile, dye decolorization is a metabolic progression; the alteration in temperature causes a modification from the ideal result into a deterioration of dye decolorization. The dying industries had different temperature zones, and sometimes increased temperatures, and the organism *Priestia megaterium* could decolorize 84.4% of azo dyes at a temperature of 45ºC. If an increase in temperature occurs, the treatment should be conducted at the optimum temperature. A comparable outcome of temperature was achieved by *Bacillus subtilis* in crystal violet dye filth (Kochher & Kumar 2011). Lalnunhlimi & Krishnaswamy (2016) described that bacterial cultures exhibit optimum dilapidation effectiveness through temperature ranges of 30-35ºC. Another researcher observed that the optimum temperature for the decolorization of

Table 6: The effect of pH on azo dye degradation by *Priestia megaterium*.

MS Broth	pH	Initial absorbance	Final absorbance	Percentage of decolorisation
	3.5	1.24	0.92	25.8
	4.5	1.22	0.53	56.5
	7	1.26	0.00	100
	8.5	1.23	0.04	96.7
	9.5	1.22	0.10	92

Table 7: The effect of temperature on azo dye degradation is *Priestia megaterium*.

MS Broth	Temperature °C	Initial absorbance	Final absorbance	Percentage of decolorisation
	15	1.24	0.61	50.8
	25	1.23	0.22	82.1
	35	1.24	0.00	100
	45	1.22	0.19	84.4
	55	1.24	1.20	3.2

Table 8: The effect of glucose concentration on azo dye degradation by *Priestia megaterium*.

MS Broth	Glucose Concentration	Initial absorbance	Final absorbance	Percentage of decolorisation
	1	1.24	0.56	54.8
	1.5	1.22	0.29	76.2
	2	1.23	0.03	97.6
	2.5	1.24	0.80	30.6
	3	1.24	0.89	28.2

malachite green was achieved via an innovative enzyme from *Bacillus cereus* Wanyonyi et al. (2017). Similarly, in Bishnoi et al. (2024), the optimal temperature for the degradation of phenanthrene was observed at 30°C, whereas biodegradation efficiency decreased as temperature increased or decreased.

Optimization of Glucose Concentration

There is an optimal pH for the organism. D20 is between 7 and 8.5; the optimum temperature for D20 is between 35 and 45, and the optimum glucose concentration is between 0.5 and 1. The maximum degradation of azo dye was observed at 0.15 a concentration Table 8. At this glucose concentration, the maximum decolorization was about 97.6%.

Application of Studies

Cotton roll and gauze roll decolorization: Cotton and gauze rolls in minimal salt broth amended with 5% dye absorbed the color in one hour, changing their color to deep purple. The medium inoculated with *Priestia megaterium* for 24 hs showed decolorization of cotton and gauze rolls. Another flask with cotton and gauze rolls without adding *Priestia megaterium* was kept as a control for the study (Fig. 9).

From the experiment, it was observed that the cotton roll was more decolorized than the gauze roll. Gauze roll is the same as cotton stuff. This experiment showed that the *Priestia megaterium* can decolorize the dye in cloths. Similar works done by Thirupathi et al. (2021) also found similar results, where nine ligninolytic microbial strains were isolated from loam samples. Entirely the strains were talented enough to decolorize a blend of azo dyes (DR 23, DB 15, and DY 12). Tiercelatent strains with extreme dye decolorizing proficiency were designated and identified by 16S rRNA gene sequencing studies. The ligninolytic microbial consortium WGC-D was found to be extra-operative,

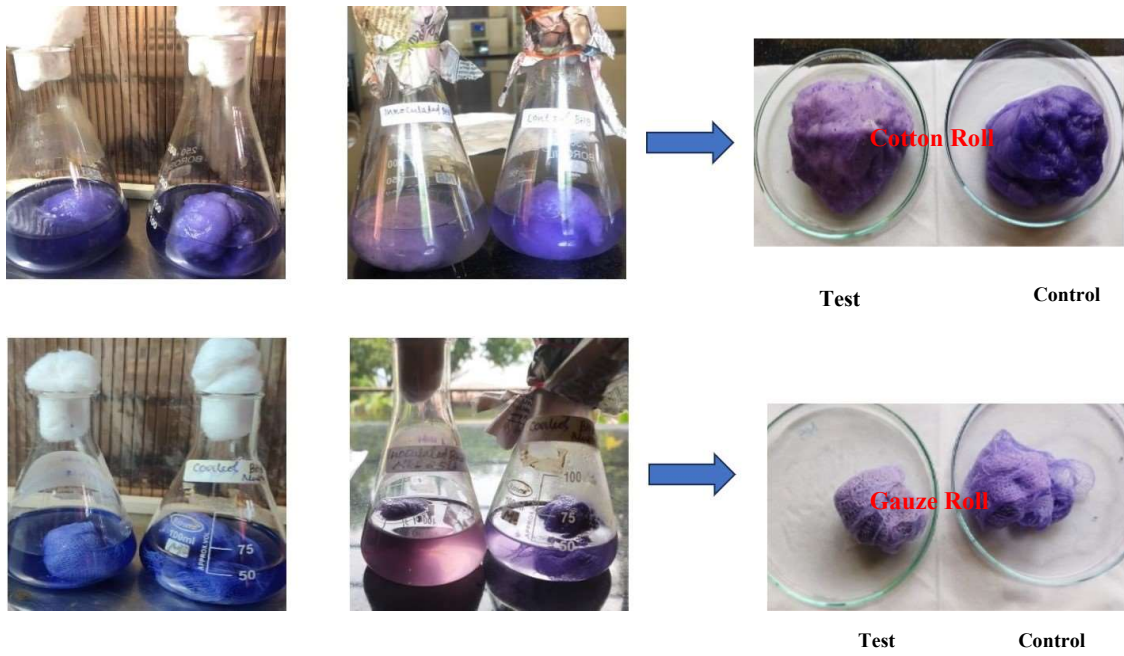

Fig. 9: Cotton roll and gauze roll decolourization.

Table 9: Cotton Roll and Gauze Roll Decolourization.

Minimal salt broth with 5% of dye	Cotton Roll Decolourization OD 410 nm	Gauze Roll Decolourization OD 410 nm
Initial Absorbance	1.25	1.25
Final Absorbance	0.09	0.10
% of decolorization	92.8	92

which decolorized up to 75% of the dye represented in Table. 9.

Bioremediation Studies

The 15 pots planted with 10 okra seeds each were keenly observed for germination for a period of one week. Each day's result was recorded, and the number of seeds that sprouted was checked. The result emphasized the possibility of *Priestia megaterium* as a growth-promoting bacteria.

The results from the study indicate that *Priestia megaterium* has growth-promoting activity, which is helping in the seed germination process. The excellent growth in the presence of *Priestia megaterium* in the soil with dye indicates the presence of nutrients, which can be intermediate metabolites produced by the bacteria, as represented in Fig. 10.

Similar works were conducted by Ebency et al. (2013), who germinated different plants, such as green gram, kidney beans, and fenugreek, with *Bacillus* sp. Nope, phytotoxicity was pragmatic for the attentiveness of the dye castoff in the study. Upright sprouting and shoot, root length of the floras was experiential for both dye and tainted dye unprotected kernels, subsequently likening with control. The isolate from the seepage liberation site exposed latent debasing dyes in quicker proportion with a bid of virtuous seed sprouting proficiency.

Set-1: Soil + Seed = Seeds showed normal growth.

Set-2: Soil + Seed + Organism = A healthier growth than normal growth in the presence of *Priestia megaterium* was observed.

Set-3: Soil + Seed+ Dye (6%) = no seed germination even after 6 days.

Set-4: Soil + Seed + Dye (6%) + Organism = All the seeds were germinated and showed maximum growth in terms of shoot length

Set-5: Soil + Seed + Dye (8%) + Organism = seed

Fig. 10: Germination studies for bioremediation.

germination was observed for 7-8 seeds, and the seedlings produced were in good health

CONCLUSIONS

Azo dyes are now crucial in a variety of businesses, particularly as consumers' preferences are heavily influenced by color. However, as azo dye use has increased, several health and environmental issues have surfaced, some of which are brought on by azo dyes and their metabolites. Bioremediation should be utilized since contemporary civilizations strive for environmentally friendly solutions. Microorganisms have demonstrated adaptable performance not only in the biomedical field but also in the application areas of the environment. There is a lot of untapped potential in microorganisms, and numerous enzymes from various microbes need to be further examined. Also important to consider is how well these microbes can tolerate a variety of xenobiotics. Over the years, modern technology has made enormous strides.

In conclusion, the isolation, identification, and partial characterization of *Priestia megaterium* strain NRBC 15308 as a potential dye-degrading bacterium provides valuable insights into sustainable environmental practices. The utilization of this bacterium in bioremediation efforts holds promise for effectively addressing dye contamination in soil while promoting eco-friendly solutions for a cleaner and healthier environment.

Priestia megaterium strain NRBC 15308 might be castoff as an auspicious for real-world bids in decolorizing and concurrently lessening the harmfulness of textile dyes. The metabolites molded after the decolorization of dye are prerequisites to be analyzed; therefore, they can be used as castoffs as admirable bio-agents for the biodegradation of textile dyes. As we move towards a more sustainable future, such studies play a crucial role in fostering responsible environmental stewardship.

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