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Original Article

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Textile Dye Decolorization Potential of *Bacillus pumilus* and *Staphylococcus saprophyticus* Isolated From Textile Industry Effluents

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Textile dyeing industries are usually chastised for being big polluters due to the poisonous nature of most dyes, which endangers all kinds of life, including people. In this study, dye degrading bacteria were isolated from water and soil samples contaminated with textile dye taken from Batik palli in Narayanganj and the ability of five isolated bacteria including *Staphylococcus saprophyticus*, *Bacillus pumilus*, *Micrococcus endophyticus*, *Pseudomonas mendocina*, and *Acinetobacter baumannii*, to decolorize crystal violet (CV) dye was investigated. Among these, *Bacillus pumilus* decolorized 58% of CV at 250 ppm, while *Staphylococcus saprophyticus* decolorized 48% of CV, after 3 days of incubation at 37°C. We examined multiple temperature and pH conditions to determine the best parameters for CV dye decolorization. *Bacillus pumilus* boosted decolorization rates by 65.39% at 37°C and at pH 7.0, while *Staphylococcus saprophyticus* increased decolorization rates by 58.73%, at 37°C and at pH 5.0. Furthermore, extending the incubation period to 6 days enhanced decolorization rate in both isolates, with *Bacillus pumilus* increased from 58% to 65% and *Staphylococcus saprophyticus* increased from 48% to 58%. Nevertheless, the inclusion of co-substrates such glucose and yeast extract further boosting decolorization rate for both isolates, approximately tripling it. As a result, this study discovered indigenous bacteria capable of decolorizing CV dye, implying that they could be employed in the treatment of textile wastewater effluents.

Keywords: Crystal violet dye, bacteria, dye decolorization

Introduction

Over the last ten years, Bangladesh has steadily industrialized, assisting the country's economy to grow. Nonetheless, many of these industries are located along the banks of major rivers and discharge untreated trash into the water, severely contaminating the environment and endangering people and other living things. Many synthetic dyes used in the textile, food, plastic, paper, and cosmetic industries are among the major pollutants causing river pollution in Bangladesh [1]. The heavy chemical coating and color of water severely limit light transmission and photosynthesis rates, putting aquatic life and biodiversity at risk. The migration of this effluent to nearby fields reduces soil productivity due to the dyes' pore-clogging effects and the hardening of the soil's texture, which limits root penetration [2]. Humans who come into touch with these dye-containing effluents have dermatitis, nasal septum perforation, and severe respiratory tract irritation [3].

Most synthetic dyes are poisonous and resistant to degradation due to their complicated chemical composition. Among the diverse classes of synthetic dyes used in industries, triphenylmethane dyes are the most numerous and flexible group of dyes in a wide range of industrial applications. Crystal Violet (CV) is a triphenylmethane dye that has been widely utilized as a biological stain in human and veterinary medicine, as well as a textile dye to give a deep violet hue [4]. Despite its numerous applications, CV is resistive and lingers in the environment for an extended period of time [5]. It functions as a mitotic toxin, a severe carcinogen, and a powerful clastogenic, increasing tumor formation in several fish species. Because of the dye's basic nature, it produces minor eye irritation, uncomfortable light sensitivity, and irreversible damage to the cornea and conjunctiva, and in severe cases, it can result in respiratory and kidney failures [6]. CV is thus classified as a biohazardous substance [7].

In Bangladesh, very few textile enterprises have sufficient effluent treatment facilities, and those that do frequently use a combination of physical and chemical procedures such as vacuum evaporation, photocatalysis, chloride bleaching, membrane filtration, and so on. Nonetheless, these methods are expensive and generate a lot of sludge, which pollutes the land. This led the search for alternative effluent treatment approaches, and in recent years, microbial bioremediation of textile dye effluents has piqued global interest due to its low cost, environmental friendliness, and capacity to treat a wide range of effluents [2]. Previous research has discovered that various bacterial and fungal species, including *Bacillus* spp.[8], *Enterobacter* spp. [2], *Pseudomonas putida* [5], *Staphylococcus saprophyticus* [9], *Aspergillus niger* [10], white rot fungus can

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degrade crystal violet [11]. Therefore, the goal of this work was to isolate indigenous bacterial species from Bangladesh that have the potential for dye decolorization and/or degradation and might be used to treat textile industry effluent water in Bangladesh.

Materials and Methods

Materials and reagents

The textile dye, Crystal Violet dye was purchased from Merck, Germany. The Bushnell Hass medium (KH_2PO_4 – 0.1%, K_2HPO4 – 0.1%, $MgSO_4$. $7H_2O$ – 0.02%, $CaCl_2.2H_2O$ – 0.002%, NH_4Cl – 0.1%, NH_4NO_3 – 0.1%, NaCl – 0.01%, and $FeCl_3$. $6H_2O$– 0.005%; pH 7.0) supplemented with 250ppm crystal violet (CV) was used for isolation of dye decolorizing bacteria.

Isolation, screening and identification of dye-decolorizing bacteria

Soil and water that had been contaminated by textile dye-containing effluents released during the washing of dyed fabric at a local Batik Palli in Narayanganj, Bangladesh, were collected. The dye-decolorizing bacteria were isolated in Bushnell Hass medium enriched with CV dye. The bacterial isolates were identified using 16s rDNA sequencing. The partial 16S rDNA gene sequence of the examined bacteria was evaluated in GenBank (NCBI) using a nucleotide BLAST search using universal 16s rDNA primer, 27 forward and 1492 reverse primer [12].

Dye decolorization assay

For the dye decolorization assay, Erlenmeyer flasks (250/ mL) containing 50/ mL of sterilized Bushnell Haas medium (pH 7.0) supplemented with CV dye to a final concentration of 250 ppm were inoculated with 10% (v/v) inoculums of each isolate and incubated for 6/ days (at 37/ °C with 150/ rpm) [13]. Control was maintained in the absence of inoculation. The decolorization of CV was monitored in terms of a decrease in color intensity by measuring absorbance at 590 nm at regular time intervals from the third to sixth day of incubation. After that the culture broth was centrifuged (10,000g, 15 min at 4°C) and the absorbance of the supernatant was recorded using an OPTIMA SP-300 spectrophotometer. The decolorization activity was estimated as a percentage of decolorization using the formula below [14].

$$\%\ Decolorization = \frac{(InitialOD - FinalOD)}{InitialOD} \times 100$$

Effect of physicochemical parameters on dye decolorization

Effect of co-substrates on dye decolorization

In dye decolorization investigations, glucose and yeast extract are widely utilized as co-substrates [13]. Hence, 0.3% Glucose and 0.125% yeast extract were added to Bushnell Haas medium (pH 7.0) supplemented with CV dye to a final concentration of 250 ppm to study the effect of these co-substrates on dye decolorization. The dye decolorization experiment was carried out exactly as described previously. As a control, Bushnell Haas medium without co-substrates was utilized.

Effect of incubation time on dye decolorization

Erlenmeyer flasks (250/ mL) containing 50/ mL of sterilized Bushnell Haas medium (pH 7.0) supplemented with CV dye to a final concentration of 250 ppm and containing 0.3% glucose and 0.125% yeast extract were inoculated with 10% (v/v) inoculums of *Bacillus pumillus or Staphylococcus saprophyticus* and incubated for 6/ days (at 37/ °C with 150/ rpm) [13]. Control was maintained in the absence of inoculation. To evaluate the influence of incubation period, samples were collected at regular intervals from the third to sixth day of incubation, and dye decolorization was measured as described before.

Effect of incubation temperature on dye decolorization

Experiments with crystal violet decolorization were carried out at two different temperatures (30 and 37°C). Fifty milliliters of sterile Bushnell Haas medium (pH 7.0) supplemented with CV dye (250 ppm), 0.3% glucose, and 0.125% yeast extract were inoculated with 10% (v/v) *Bacillus pumillus* or *Staphylococcus saprophyticus* inoculums and incubated at the appropriate temperatures. In the absence of inoculation, control was maintained. Dye decolorization was evaluated in the same manner as previously described.

Effect of incubation pH on dye decolorization

To investigate the effect of pH on dye decolorization, Bushnell Haas medium supplemented with CV dye (250 ppm) and 0.3% Glucose and 0.125% yeast extract were prepared and the pH of the medium was adjusted to 3, 5 or 7 using 0.1 N HCl and 0.1 N NaOH.

Statistical analysis

The experiments were conducted in triplicate and mean ± standard deviation values were expressed while Graph Pad Prism Software (GPPS 8) was used for data analysis.

Results

Isolation and identification of CV decolorizing bacterial isolates

Following inoculation of samples on Bushnell Hass medium supplemented with CV, five different types of bacterial colonies were obtained. The findings of the Gram staining and biochemical tests did not provide a definitive decision on the identification of the isolates. Based on the analysis of 16s rDNA sequencing data the isolates were identified as *Bacillus pumillus, Micrococcus endophyticus, Acinetobacter baumannii*, *Pseudomonas mendocina,* and *Staphylococcus saprophyticus* (Table 1).

Table 1: *The summary of the blast result*

Isolates	Organism	Gene bank accession number
1	*Bacillus pumillus*	MZ676076.1
2	*Micrococcus endophyticus*	MZ676078.1
3	*Acinetobacter baumannii*	MZ820115.1
4	*Pseudomonas mendocina*	MZ820116.1
5	*Stenotrophomonas acidaminiphila*	MZ820117.1
6	*Staphylococcus saprophyticus*	MZ676075

Dye decolorization by bacterial isolates

Staphylococcus saprophyticus and *Bacillus pumilus* were chosen for the dye decolorization assay based on preliminary data. At a final dosage of 250 ppm, these two isolates were examined for their capacity to decolorize CV. *Bacillus pumilus* and *Staphylococcus saprophyticus* decolorized 58% and 48% CV after 3 days of incubation at 37°C, respectively (Figure 1).

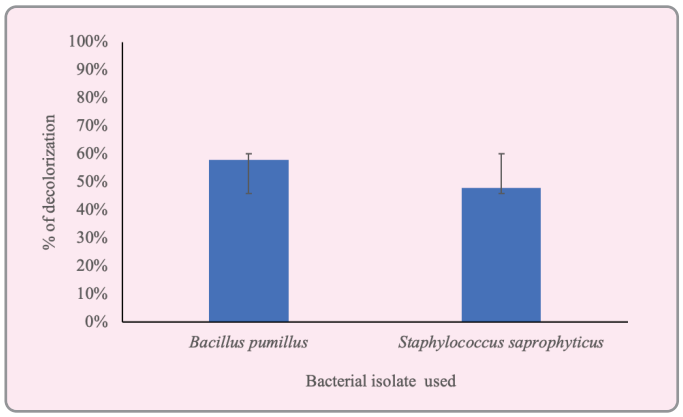

Figure 1: *Decolorization assay of crystal violet at 250 ppm for 3 days by Bacillus pumilus and Staphylococcus saprophyticus.*

Effect of co-substrates on dye decolorization

The presence of co-substrates glucose and yeast extract influenced CV decolorization. Adding glucose and yeast extract to *Bacillus pumilus* and *Staphylococcus saprophyticus* improved decolorization almost three-fold (Figure 2). As a result, these co-substrates were used in all subsequent tests.

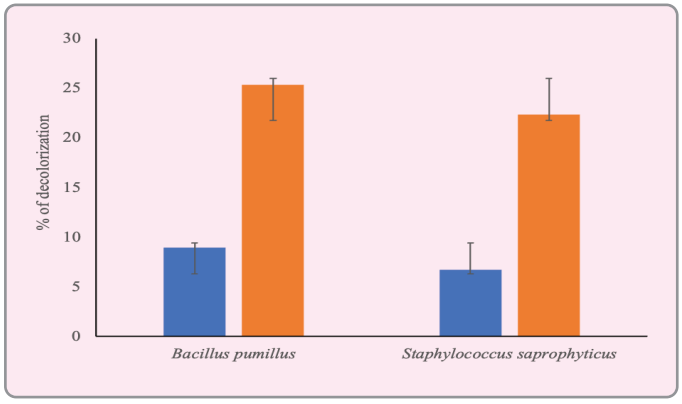

Figure 2: *Effect of co-substrates on crystal violet decolorization by Bacillus pumilus and Staphylococcus saprophyticus. Blue bar indicates absence of co-substrates and orange bar indicates addition co-substrates.*

Effect of incubation time on dye decolorization

The decolorization assay was performed for 3 and 6 days with both *Bacillus pumilus* and *Staphylococcus saprophyticus* to determine how incubation time effects crystal violet decolorization. The results showed that increasing the incubation days from 3 to 6 days boosted crystal violet decolorization from 58% to 65% for *Bacillus pumilus* and 48% to 58% for *Staphylococcus saprophyticus* (Figure 3).

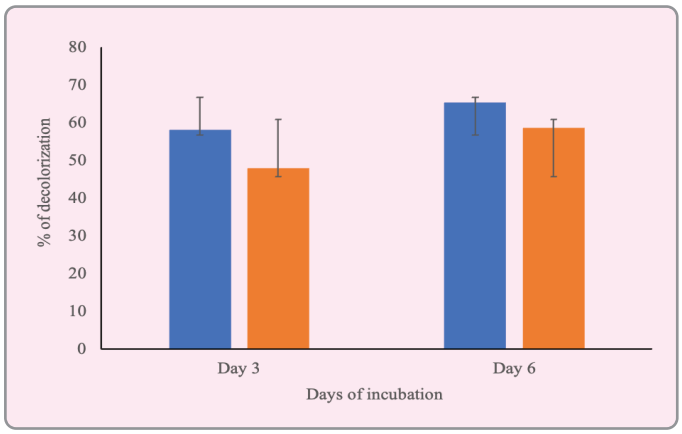

Figure 3: *Effect of incubation period on crystal violet decolorization by Bacillus pumilus and Staphylococcus saprophyticus. Blue bar indicates Bacillus pumilus and orange bar indicates Staphylococcus saprophyticus.*

Effect of incubation temperature on dye decolorization

The decolorization assay was done at 30°C and 37°C to further investigate the optimal physicochemical conditions for CV decolorization by *Bacillus pumilus* and *Staphylococcus saprophyticus.* Figure 4 shows that at 30°C, dye decolorization by *Bacillus pumilus* and *Staphylococcus saprophyticus* was around 7% and 22%, respectively. Both isolates showed significantly increased decolorization at 37°C.

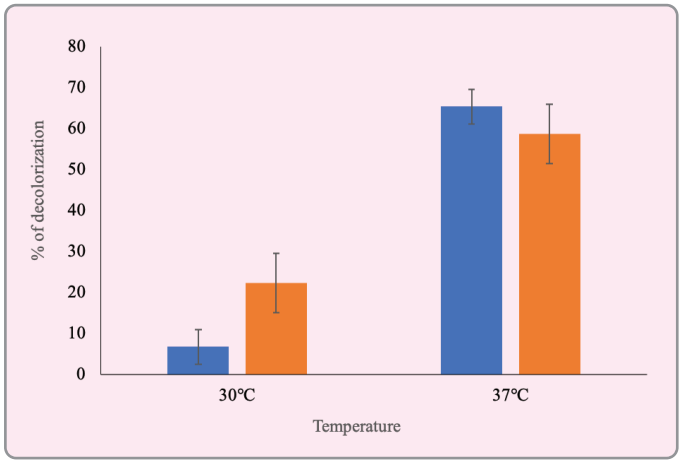

Figure 4: *Effect of temperature on crystal violet decolorization by Bacillus pumilus and Staphylococcus saprophyticus. Blue bar indicates Bacillus pumilus and orange bar indicates Staphylococcus saprophyticus.*

Effect of pH on dye decolorization

To study the ideal physicochemical parameters for CV decolorization by *Bacillus pumilus* and *Staphylococcus*

saprophyticus, the decolorization assay was done at pH 3, 5, and 7. *Bacillus pumillus* decolorized Crystal Violet (CV) the fastest in neutral pH conditions, specifically pH 7. However, *Staphylococcus saprophyticus* decolorized more efficiently at somewhat acidic pH levels, particularly pH 5 (Figure 5). The differing pH preferences reported in the two bacterial strains imply that the ideal physicochemical parameters for CV decolorization may differ between microorganisms.

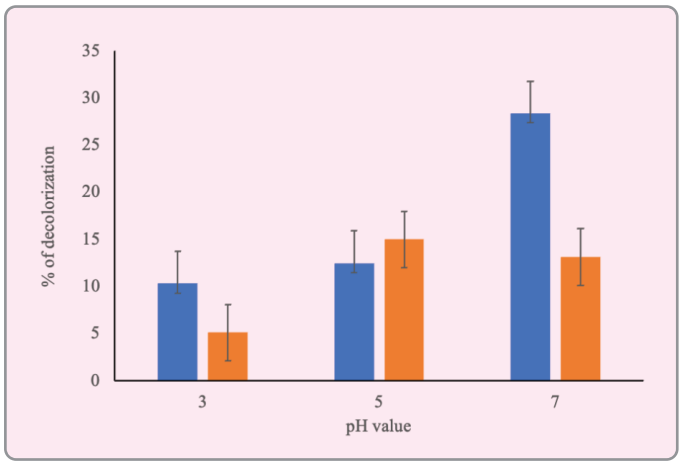

Figure 5: *Effect of pH on crystal violet decolorization by Bacillus pumilus and Staphylococcus saprophyticus. Blue bar indicates Bacillus pumilus and orange bar indicates Staphylococcus saprophyticus.*

Discussion

Industrial wastewater treatment has been a global concern due to the environmental risks of present physical and chemical technologies. Microorganism-based bioremediation of toxic waste residues is a feasible alternative to physicochemical techniques. As a result, the current study isolated and identified five indigenous bacteria from textile dye effluent contaminated water collected in Narayanganj, Dhaka, in order to develop a cost-effective bioremediation waste treatment method for removing harmful, mutagenic dye from water.

The isolates were identified as *Staphylococcus saprophyticus, Bacillus pumilus, Micrococcus endophyticus*, *Pseudomonas mendocina* and *Acinetobacter baumannii.* The ability of all of these bacteria to decolorize dye is well documented, since many other researchers have reported the participation of these bacterial genera in diverse dye degradation investigations. Several studies reported *Pseudomonas mendocina* [15], *Staphylococcus saprophyticus* [16], *Acinetobacter baumannii* [17,18] as potent textile dye degrading bacteria. Among the isolates obtained, *Bacillus pumilus* and *Staphylococcus saprophyticus* showed potential for decolorization of CV. In experiments conducted in Bangladesh [9,13], *Staphylococcus saprophyticus* was found to be a strong dye decolorizer, decolorizing 94% of methyl red after 24 hours and 65% of Benzema yellow S8-G after 6 days. While *Bacillus pumilus* was found to completely degrade Triazo Acid Black 210 (AB210) dye with a dye concentration of 100 mg/L in 85 minutes under ideal conditions [19], another study discovered that it decolored 17% of Direct Red 81 (DR81) dye with a dye concentration of 100 mg/L after 5 days at 300.2°C under aerobic conditions [20]. This suggests that the bacteria isolated in the current investigation have been found in earlier studies and have actual dye decolorization capabilities. The current study's findings emphasized the importance of co-substrates in promoting CV decolorization by *Bacillus pumilus* and *Staphylococcus saprophyticus*, as has been documented in other research [8]. The addition of glucose and yeast extract as co-substrates increased decolorization effectiveness by nearly double it. The observed improvement in CV decolorization can be related to the involvement of glucose and yeast extract as supplemental nutrients, which provide the bacteria with critical carbon and energy sources, meaning that the bacteria operated at suboptimal metabolic rates when they were not present. Similar results were obtained from a research of the decolorization of the Indanthrene Blue RS dye bacterial consortium, which showed that when carbon and nitrogen supplies were employed as supplements, consortium-BP produced a greater decolorization efficiency of the dye[21].

The results demonstrated that increasing the incubation time from 3 to 6 days enhanced the ability of both bacterial strains to decolorize Crystal Violet dye. This shows that because the bacteria had more time to break down and metabolize the dye molecules over the longer incubation period, the decolorization rates were higher. A comparable study discovered 90% decolorization of Novacron Brilliant Blue FN-R and Novacron Super Black G dyes by bacterial consortium in the presence of co-substrates after 6 days[13]. In another study, moderately alkaliphilic bacterial consortia decolored the dyes Direct Blue 151 (DB 151) and Direct Red 31 (DR 31) in under 5 days [22].

The temperature of incubation differs amongst microorganisms and has a major impact on their development and enzyme activity. In this investigation, the dye decolorization rates of the bacterial isolates were greater at 37°C, showing that the optimal temperature promotes more efficient decolorization. A related investigation discovered that B. cereus destroyed CV (500 ppm) in 2.5 hours under shaking conditions at pH 7 and 30 °C [8]. Another investigation found that Enterobacter sp. CV-S1 completely decolorized CV after 72 hours of shaking incubation at 35 °C [2]. Because dye decolorization is a physiological process, temperature changes reduce it. This knowledge is critical for understanding how the appropriate temperature influences these bacteria' ability to decolorize, which is vital for potential bioremediation applications in dye-contaminated environments.

The pH level is an important component in the degradation of textile colors by microbes. *Bacillus pumilus* decolorized the most at pH 7 (neutral), while *Staphylococcus saprophyticus* decolorized the most at pH 5 (slightly acidic). Similarly, in the presence of co-substrates, *Aeromonas hydrophila* decolorizes 99% CV at pH 7 at 35°C [23]. In another work, Enterobacter sp. CV-S1 destroyed

99% CV in a slightly acidic (pH 6.5) environment [2]. Pseudomonas sp. SUK1 decolorized sulfonated azo dye (Reactive Red 2) at pH range 6.2 - 7.5 in another work [24]. As a result of the different pH preferences observed in the two bacterial strains, the optimal physicochemical parameters for CV decolorization may differ between microorganisms, emphasizing the importance of taking pH into account when designing or optimizing decolorization processes for different bacterial species.

Textile dyeing effluents are a major source of water pollution in Bangladesh, and cleanup is a difficult process. So, among the indigenous bacterial isolates recovered and identified from textile effluents, *Staphylococcus saprophyticus* and *Bacillus pumilus* were determined to have the best potential for CV dye degradation in the current investigation. They can successfully degrade CV when the temperature, pH, incubation period, and appropriate co-substrates are optimized.

Thus, this study looked into the bacterial bioremediation of dye-contaminated harmful industrial wastewater, which could be a more sustainable alternative to chemical treatment approaches. The microorganisms discovered in this study show great promise for being exploited to develop a cost-effective and environmentally friendly bioremediation approach for the detoxification and breakdown of synthetic colors. To promote the use of these isolates as prospective bioremediation agents, more study on the mechanisms involved in dye decolorization, enhancement of their cultural conditions, and molecular characterization are required.

Acknowledgment

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References

1. Lellis, B., Fávaro-Polonio, C. Z., Pamphile, J. A. & Polonio, J. C. Effects of textile dyes on health and the environment and bioremediation potential of living organisms. *Biotechnology Research and Innovation* **3**, 275–290 (2019).
2. Roy, D. C. *et al.* Biodegradation of Crystal Violet dye by bacteria isolated from textile industry effluents. *PeerJ* **6**, e5015 (2018).
3. Ghaly, A. E., Ananthashankar, R., Alhattab, M. & vasudevan ramakrishnan, V. Production, characterization and treatment of textile effluents: A critical review. *J. Chem. Eng. Process. Technol.* **5**, (2014).
4. Ashraf, M. W., Collins, M. P., Mir, M. A., Salam, A. & Ul-Hamid, A. Separation of Crystal Violet Dye from Wastewaters by Using Supported Liquid Membrane Technology. *Journal of Chemistry* **2022**, e8143981 (2022).
5. Chen, C.-C., Liao, H.-J., Cheng, C.-Y., Yen, C.-Y. & Chung, Y.-C. Biodegradation of crystal violet by Pseudomonas putida. *Biotechnol Lett* **29**, 391–396 (2007).
6. Mani, S. & Bharagava, R. N. Exposure to Crystal Violet, Its Toxic, Genotoxic and Carcinogenic Effects on Environment and Its Degradation and Detoxification for Environmental Safety. *Rev Environ Contam Toxicol* **237**, 71–104 (2016).
7. Mirza, A. & Ahmad, R. An efficient sequestration of toxic crystal violet dye from aqueous solution by Alginate/Pectin nanocomposite: A novel and ecofriendly adsorbent. *Groundwater for Sustainable Development* **11**, 100373 (2020).
8. Ayed, L., Cheriaa, J., Laadhari, N., Cheref, A. & Bakhrouf, A. Biodegradation of crystal violet by an isolatedBacillus sp. *Annals of Microbiology* **59**, 267 (2009).
9. Hakim, A., Hoque, S. & Ullah, S. Decolorization of Methyl Red by Staphylococcus saprophyticus strain AUCASVE3 Isolated from Textile Effluent. *EnvironmentAsia* **7**, 32–38 (2014).
10. Ali, H., Shehata, S. & Ramadan, K. Microbial decolorization and degradation of crystal violet dye by Aspergillus niger. *International Journal of Environmental Science and Technology* **13**, (2016).
11. Bumpus, J. & Brock, B. Biodegradation of Crystal Violet by the White Rot Fungus Phanerocheate chrysosporium. *Applied and environmental microbiology* **54**, 1143–50 (1988).
12. Frank, J. A. *et al.* Critical Evaluation of Two Primers Commonly Used for Amplification of Bacterial 16S rRNA Genes. *Applied and Environmental Microbiology* **74**, 2461–2470 (2008).
13. Karim, M. E., Dhar, K. & Hossain, M. T. Decolorization of Textile Reactive Dyes by Bacterial Monoculture and Consortium Screened from Textile Dyeing Effluent. *J Genet Eng Biotechnol* **16**, 375–380 (2018).
14. Cheriaa, J., Khaireddine, M., Rouabhia, M. & Bakhrouf, A. Removal of Triphenylmethane Dyes by Bacterial Consortium. *ScientificWorldJournal* **2012**, 512454 (2012).
15. Sarnaik, S. & Kanekar, P. Biodegradation of methyl violet by Pseudomonas mendocina MCM B-402. *Appl Microbiol Biotechnol* **52**, 251–254 (1999).
16. Kumari, L. *et al.* Biodegradation of Navy N5RL1 carpet dye by Staphylococcus saprophyticus strain BHUSS X3. *3 Biotech* **5**, 775–782 (2015).
17. Ameenudeen, S., Unnikrishnan, S. & Ramalingam, K. Statistical optimization for the efficacious degradation of reactive azo dyes using Acinetobacter baumannii JC359. *J Environ Manage* **279**, 111512 (2021).
18. Unnikrishnan, S., Khan, M. H. & Ramalingam, K. Dye-tolerant marine Acinetobacter baumannii-mediated biodegradation of reactive red. *skxysgc* **11**, 265–275 (2018).
19. Agrawal, S., Tipre, D. & Dave, S. Biodegradation of Sulphonated Triazo Acid Black 210 Dye by Thermohalotolerant Bacillus pumilus SRS83: Kinetic, Metabolite and Toxicity Analysis. *Appl Biochem Microbiol* **58**, 613–626 (2022).
20. Al-Shareef, W. A., Afifi, S. S. I., Ramadan, M. A. & Sakr, N. R. Improvement of the Decolorization of Azo Dye (Direct Red 81) by Immobilized Cells of Bacillus pumilus and Free Cells of Aspergillus clavatus in Textile Waste Waters. *Int.J.Curr.Microbiol.App.Sci.* **7**, 1690–1707 (2018).
21. Mohanty, S. S. & Kumar, A. Enhanced degradation of anthraquinone dyes by microbial monoculture and developed consortium through the production of specific enzymes. *Sci Rep* **11**, 7678 (2021).
22. Lalnunhlimi, S. & Krishnaswamy, V. Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium. *Brazilian Journal of Microbiology* **47**, 39–46 (2016).
23. Bharagava, R. N., Mani, S., Mulla, S. I. & Saratale, G. D. Degradation and decolourization potential of an ligninolytic enzyme producing Aeromonas hydrophila for crystal violet dye and its phytotoxicity evaluation. *Ecotoxicol Environ Saf* **156**, 166–175 (2018).
24. Kalyani, D. C., Telke, A. A., Dhanve, R. S. & Jadhav, J. P. Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated Pseudomonas sp. SUK1. *Journal of Hazardous Materials* **163**, 735–742 (2009).