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Evaluation of the Degradation of Textile Dyes by Bacterial Strains Isolated from Mangroves in Cartagena, Colombia

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Abstract*– Dyes are widely used in various industries, but they represent significant pollutants due to their harmful effects on the environment. Their high solubility facilitates their dispersion and complicates their removal in aqueous media. In addition, intermediate compounds such as naphthalene and benzene, present in some dyes, are highly toxic to various biological systems. Bioremediation with microorganisms is an effective, simple and sustainable alternative to address this problem. This study evaluated the ability of bacterial strains isolated from Cartagena mangroves (UTB 24, UTB 35, UTB 44, UTB 63, UTB 67 y UTB 85), related to the Bacillus genus, to degrade textile dyes. The strains were grown in MBS medium with 1000 mg/L of dye, incubated at 30 °C for 48 hours, and the percentage of degradation was determined. Most strains showed a degradation greater than 60%, with strains UTB 24, UTB 35, UTB 63 and UTB 67 standing out, which exceeded 80%. The results obtained demonstrate the potential of bacteria from mangals to be used in remediation strategies for bodies of water contaminated by dyes.

Keywords*-- Biodegradation, environmental pollution, mangroves, textile dyes.

I. Introduction

Dyes are organic aromatic molecules that form covalent bonds with the hydroxyl groups of the cotton fiber [1-2]. There are more than 20 different classes of dyes, among which the most common ones include anthraquinone, azo, triphenylmethane, azine, nitroso, nitro, phthalocyanine, thiazole, lactone, sulfur and diarylmethane [3]. Azo dyes represent the most widely used group of synthetic dyes in different industries, since the azo bond of their structure, combined with different chemical groups, generates structural diversity that gives rise to a wide variety of colours [2].

The annual production of these dyes is estimated to be approximately 7×105 metric tons, however, between 15 - 20% is lost during production and processing, and is released without any adequate treatment into the environment [4]. Reducing light penetration into water bodies can negatively impact the photosynthetic activity of aquatic organisms. In addition, these synthetic dyes have been reported to be carcinogenic, mutagenic, genotoxic and allergenic to both aquatic life and humans [2, 5].

There are different physicochemical methods for the treatment of effluents with azo dyes which include adsorption, irradiation, ion exchange, precipitation, filtration, coagulation-flocculation, ozone treatment and electrochemical methods [6-7]. However, these methods have limitations due to their high cost, operational difficulties, large energy requirements, time demand, production of hazardous by-products and generation of toxic sludge [1, 8-9]. Therefore, it is essential to develop bleaching methods that are economical, non-toxic and environmentally friendly.

Bioremediation, which takes advantage of the metabolic potential of bacteria, fungi or plants, is an effective and environmentally friendly alternative that can be used for the treatment of wastewater contaminated with azo dyes [2,10]. Biological methods offer advantages over conventional treatments, including simplicity, cost-effectiveness, complete mineralization, and minimal production of toxic sludge. In addition, the use of organisms for contaminant removal is more flexible as it can be used both in-situ and ex-situ [2,11]. Therefore, exploration of extreme environments such as ocean, deserts, polar areas and mangroves have become an alternative for the isolation of new and effective bleaching bacteria. Particularly, mangrove ecosystems have great potential for the discovery of dye-degrading bacteria, due to their properties of high salinity, high temperature, and anaerobic and muddy soils [12]. In addition, mangrove soil is considered to act as a natural trap to retain pollutants, such as dyes and associated chemicals, which are released by local residents, community enterprises and textile industries [1, 13].

Mangroves are distributed between the sea and land in tropical and subtropical areas and are considered one of the most productive ecosystems in the world [14]. In Colombia, mangroves are distributed on the Pacific coast (282,835 ha) and the Caribbean coast (88,244 ha). Previous studies have reported the isolation of dye-decolorizing bacteria isolated from mangroves which are related to the genera *Lysinibacillus* sp. [15], *Paenibacillus* sp. [16] and *Roseobacter* sp. [17]. However, information on bacteria with the potential to degrade dyes from Colombian mangroves is limited. Therefore, the objective of this study was to evaluate the biodegradation capacity of bacteria isolated from mangroves in Cartagena, Colombia.

II. MATERIAL AND METHODS

A. Strains

In this study, the azo dye degradation capacity of 6 halotolerant aerobic bacteria previously isolated from mangroves located in Cienaga de La Virgen, Cartagena (Colombia) was evaluated [18-19]. Table 1 presents the characteristics of the selected strains.

TABLE I
CHARACTERISTICS OF STRAINS ISOLATED FROM MANGROVES IN THE CIÉNAGA DE LA VIRGEN SELECTED TO EVALUATE THE DEGRADATION OF AZO DYES

Mangrove	Sample type	Strain Code	Taxonomic afiliation
Conocarpus erectus	Roots	UTB 24	*Bacillus altitudinis*
Avicennia germinans	Stems	UTB 35	*Bacillus cereus*
Avicennia germinans	Stems	UTB 44	*Bacillus pumilus*
Avicennia germinans	Roots	UTB 63	*Bacillus haynesii*
Conocarpus erectus	Stems	UTB 67	*Bacillus australimaris*
Conocarpus erectus	Sediment	UTB 85	*Bacillus thuringiensis*

B. Dyes

The azo dyes evaluated were: Direct Blue 15, Congo Red, Malachite Green and Methyl Orange. The concentration used for the dyes evaluated was 1000 mg/L.

TABLE II
SELECTED ANTIBIOTICS AND CONCENTRATION EVALUATED

Group	Antibiotic	Concentration
β-lactams	Penicillin	100 UI
	Dicloxacillin	50 mg/mL
	Amoxicillin	50 mg/mL
	Ampicillin	50 mg/mL
Cephalosporins	Cephalexin	50 mg/mL
Sulfonamides	Sulfamethazol	50g/mL

C. Culture conditions

The strains were grown in MBS medium supplemented with each dye evaluated (1000 mg/L). The MBS medium contained ((L−1 deionised water): 1.26 g of NH4Cl, 1.71 g of K_2HPO_4, 1.32 g of KH_2PO_4, 70 g of NaCl, 0.02 g of $CaCl_2$, 0.011 g of $MgCl_2$ and 0.5 g of yeast extract [20]. The pH of the medium was adjusted to 7.0 with NaOH. The strains were subcultured twice under the same experimental conditions. The cultures were incubated at 37°C for 72 h. All tests were performed in duplicate.

D. Spectrophotometric analysis of azo dye decolorization

The decolorization of the dye was assessed using UV–VIS spectrophotometric analysis. A standard curve was made for each dye using concentrations from 100 mg/L to 1000 mg/L. Table 2 presents the wavelength and equation of the straight line for each dye evaluated.

TABLE II
WAVELENGTH AND EQUATION OF THE STRAIGHT LINE FOR THE AZO DYE EVALUATED

Dye	Wavelength	Equation of the straight line
Direct Blue 15	500 nm	0.0005x + 0.0549 R2 = 0.9423
Congo Red	600 nm	y = 0,001x + 0,0051 R^2 = 0,9946
Malachite Green	550 nm	y = 0,0217x - 0,0135 R^2 = 0,9804
Methyl Orange	400 nm	y = 0,0727x + 0,0349 R^2 = 0,9967

The cultures (1 mL) were centrifuged at 10,000 rpm for 10 min. The absorbance was determined from the supernatant of each sample. MBS medium without dye was used as a blank. The percentage of decolorization was calculated by the difference between the initial and final absorbances (Equation 1).

$$\%\ decolorization = \frac{Initial\ absorbance - Final\ absorbance}{Initial\ absorbance} \times 100\%$$

III. RESULTS

A. Decolorization of azo dyes

In this study, the potential capacity of bacterial strains isolated from mangroves in Cartagena (Colombia) to degrade azo dyes was determined. All strains evaluated showed a high percentage of malachite green dye decolorization, with percentages higher than 98%. Strain

UTB 63 showed the best performance (99.09%), while strain UTB 67 showed the lowest percentage (98.9%). The other strains presented values close to 99% (Fig. 1).

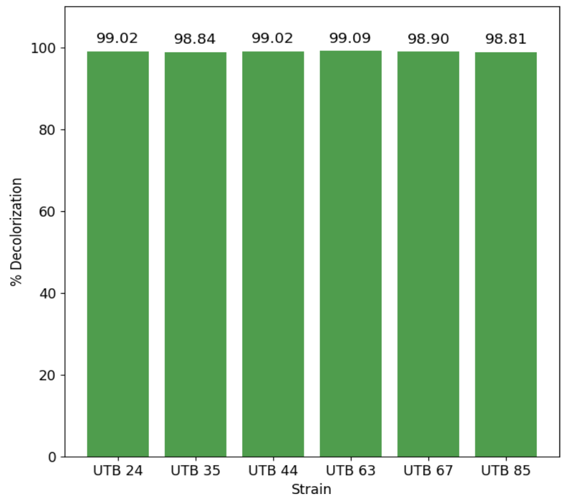

Fig. 1. Percentage of malachite green dye decolorization of the strains evaluated

The decolorization of the methyl orange dye was almost complete for all strains as well, with percentages higher than 99%. Specifically, strain UTB 24 reached 100% degradation, while the other strains ranged between 99.47% (UTB 44) and 99.85% (UTB 63) (Fig. 2).

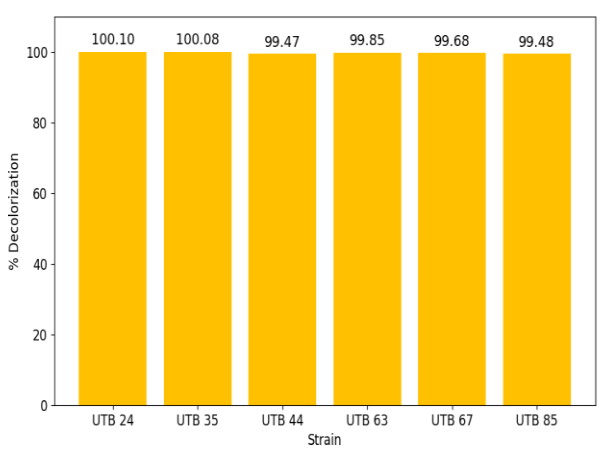

Fig. 2. Percentage of methyl orange dye decolorization of the strains evaluated

On the other hand, the strains showed different levels of efficiency in decolorizing the Direct Blue 15 dye. Strains UTB 24 and UTB 67 achieved the highest decolorization values, with percentages of 56.11% and 61.31%, respectively. In contrast, strain UTB 85 showed the lowest capacity, reaching 45.81%. Strains UTB 35, UTB 44 and UTB 63 showed intermediate values of 48.9%, 47.7% and 48.2%, respectively (Fig. 3).

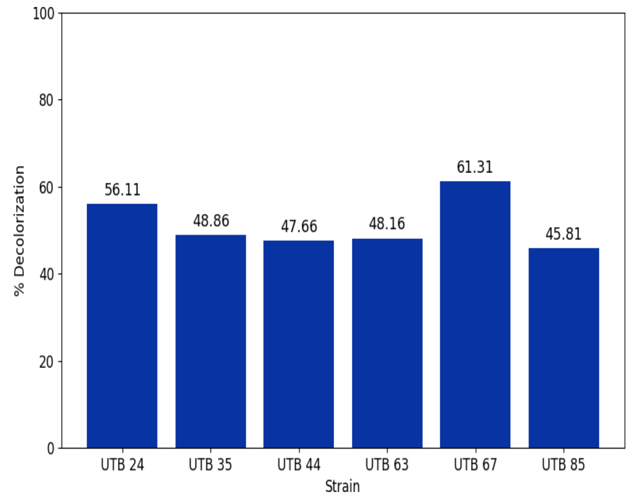

Fig. 3. Percentage of Direct Blue 15 dye decolorization of the strains evaluated

Regarding the Congo red dye, none of the strains evaluated showed the ability to decolorize, since in all cases values of 0% were reported. The results obtained in this study suggest that the bacterial strains studied have potential for applications in the bioremediation of azo dyes, particularly for malachite green and methyl orange. In addition, it was determined that strains UTB 24 (*Bacillus altitudinis*) and UTB 67 (*Bacillus australimaris*) presented the highest percentages of decolorization and have the potential capacity to degrade different types of azo dyes.

IV. Discussion

Water is one of the essential elements that sustains all forms of life, therefore, its quality is of vital importance because it is essential to support the physiological activities of any living organism [21]. Unfortunately, pollution levels have increased worldwide due to anthropogenic activities that affect water bodies such as lakes, rivers, oceans, and groundwater [22]. The textile industry is one of the main sources of water and soil pollution due to its high consumption of water and chemicals. Among the available dyes, azo dyes represent between 60 and 70% of all textile dyes produced [23]. However, during the production process approximately 10-15% of these compounds are released as effluents, further contributing to environmental pollution [24]. There are different methods for the elimination of azoic dyes, however, the persistent characteristics of dyes make them ineffective. The use of microorganisms for the elimination of dyes is an attractive alternative due to its extraordinary effectiveness, high biocompatibility, and ecological performance [25]. Therefore, in this study the potential of bacterial strains isolated from Cartagena mangroves to decolorize was evaluated.

Mangroves are ecosystems with fluctuating salinity and pH conditions, therefore, the microorganisms that inhabit these ecosystems are adapted to survive in these extreme conditions [26]. The ability of these microorganisms to tolerate extreme conditions is an attractive feature for bioremediation of sites contaminated with textile dye effluents, since most of these effluents have an alkaline pH [23]. In this study, we demonstrate that bacterial strains isolated from mangroves in Cartagena, have the ability to decolorize azo dyes. Previous studies have reported the isolation of dye-degrading bacteria from mangroves in Thailand, India and China related to the genera *Paenibacillus* [16], *Roseobacter* [17], *Lysinibacillus* [15], *Klebsiella* and *Enterobacter* [1], which demonstrates their potential use for the treatment of wastewater from the textile industry.

The bacterial strains evaluated in this study demonstrated the ability to decolorize azo dyes are related to the *Bacillus* genus. The *Bacillus* genus is made up of rod- positive, aerobic, and spore forming bacteria. This genus is found in various soil, water, and air environments. Currently, the genus has more than 250 recognized species, among which are some with nitrogen-fixing capacity, one chemolithotrophic, and several adapted to survive in extreme conditions [27]. The metabolic versatility and physiological characteristics of the *Bacillus* genus have allowed its use in various biotechnological applications, which include production of hydrolytic enzymes, bioactive compounds, biopesticides, biofungicides, and fertilizers [28]. Previous studies have reported the ability of some species of the *Bacillus* sp. to degrade different types of dyes. [29] evaluated the effect of aerobic and anoxic conditions on the biodegradation of Red B dye by *Bacillus* OY1-2 and demonstrated that this strain biodegrades this dye under both oxic and anoxic conditions. [30] evaluated the biodegradation of azo dyes using *Bacillus subtilis* and determined that this species degraded 87.35% of the mixed azo dyes (200 mg/L) in five days. Particularly in our study, strains UTB 35 and UTB 85 related to the species *B. cereus* and *B. thuringensis*, respectively, presented high percentages of decolorization (>98%) of the dyes methyl orange and malachite green. These results coincide with previous studies where the efficiency of these species to degrade azo dyes has been demonstrated. [23] investigated the biodegradation of reactive red (RR), reactive brown (RB) and reactive black (RBL) in a bioreactor using cell- free and immobilized cells of *Bacillus cereus* and identified significant degradation (86.29%) of mixed dyes with immobilized cells of this species. [31] identified that strain F5 related to the species *Bacillus thuringiensis* produces ligninolytic enzymes capable of efficiently degrading methylene blue (MB).

The differences in degradation percentages observed among dyes can be mainly attributed to their chemical structures and toxicity levels. Dyes with more complex structures or with functional groups that generate greater recalcitrance, such as azo dyes with multiple aromatic rings, tend to be more resistant to microbial action [32-33]. Furthermore, the inherent toxicity of some dyes, such as Congo Red, can negatively affect the viability or metabolic activity of degrading microorganisms, limiting their ability to transform the compound [34-35]. This would explain why, in the present study, all the strains evaluated achieved high decolorization efficiency in dyes such as Malachite Green and Methyl Orange, but did not show the ability to degrade Congo Red. Therefore, the bacterial strains showed greater efficiency in the degradation of less toxic dyes or those with more accessible chemical structures, which highlights the importance of considering both toxicity and molecular composition in bioremediation processes [3] [10]

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V. CONCLUSION

In conclusion, the present study demonstrated the ability of bacteria isolated from the mangroves of Cartagena, Colombia, to degrade azo dyes. High efficiency was observed in the decolorization of malachite green and methyl orange dyes, with percentages higher than 98% and 99%, respectively, with the UTB 63 strain standing out with the best results. However, the degradation of the Direct Blue 15 dye was variable between strains, with values between 45.81% and 61.31%. These findings highlight the biotechnological potential of these strains in the bioremediation of textile effluents.

The results obtained in this study may contribute to the environmental remediation of coastal ecosystems through the use of bacterial strains native to mangroves in Cartagena, Colombia. The high efficiency of these bacteria in the degradation of textile dyes such as malachite green, methyl orange and direct blue 15 highlights their potential for bioremediation applications. This could contribute to reducing pollution generated by the textile industry, and promote sustainable strategies based on biotechnology for the conservation of marine ecosystems.

The strains evaluated in this study were isolated from the research project "Exploration of diversity and biotechnological potential of cultivable bacteria associated with mangroves in Cartagena," which is subscribed to the Contract for Access to Genetic Resources and their Derived Products No. 333 of 2022. In addition, the strains evaluated are deposited in a certified collection of microorganisms.

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