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Article

Isolation, characterization and identification of *Klebsiella* sp. 35-DOR_27F isolated from textile effluents

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ABSTRACT

This research study aimed to isolate and characterize a new bacterial strain from textile effluents. In order to do this, bacteria were cultured using the MSM medium, where a colony was isolated through six successive pickings. It then underwent a DNA extraction process using the phenol-chloroform-isoamyl alcohol methodology, and an electrophoresis was carried out to confirm the extraction. In addition, the isolated colony was identified as *Klebsiella* sp. by sequencing the 16S rRNA gene using bioinformatics tools. To observe its biotechnological potential, the bacterial strain was grown in an MSM broth enriched with Golden Yellow K2R azoic dye at a concentration of 50 mg/L, showing a percentage of decolorization of 74 % after 72 hours at 37 °C, indicating the potential of the isolated colony for the development of bio-remediation processes for effluents containing azoic dyes.

Keywords: Bacterial strain, *Klebsiella* sp., DNA extraction, decolorization, effluents.

INTRODUCTION

One of the main problems affecting the world's environment is water contamination due to industrial activity, which generates high amounts of polluting effluents that produce toxic effects. [1]. For example, the textile industry generates wastewater containing artificial recalcitrant or carcinogenic dyes [2-4].

Worldwide, around 10,000 types of dyes and pigments are used in a wide range of diverse industries, and approximately 7×10^5 tons of them are produced annually due to the industrialization and demand of the textile industry [5,6]. Among the dyes used in the industry, around 20 % remain unused (they do not fully penetrate the fabrics) and are released directly into the sewage system. This renders the water unfit for use and impedes light penetration into bodies of surface water, adversely affecting the aquatic environment [7-9].

Industrial dyes contain various structures, most composed of three reactive groups: azo, anthraquinon and phthalocyanin [6,10,11]. Numerous research studies have been carried out to perfect a treatment that allows the degradation of these textile dyes and colorings and the recovery of the natural water resource. A viable alternative is biological treatment since it is inexpensive, ecological, and functional compared to other chemical or physical treatments that require much energy [6,11,12].

Some studies have used different bacteria for the biodegradation of reactive textile dyes, such as: *Alcaligenes faecalis*, *Bacillus cereus* and *Bacillus* sp. [6]; *Lysinibacillus sphaericus* and *Aeromonas hydrophila* [11]; *Micrococcus luteus* and *Bacillus subtilis* [4]; *Staphylococcus* sp. [13]; *Citrobacter freundii* [1]; *Planococcus* and *Bacillus* [14], *Pseudomonas* [15,16]; *Aeromonas* [17], *Bacillus* and *Lysinibacillus* [18]; *Paenibacillus polymyxa, Micrococcus luteus* and *Micrococcus* [19]. Other studies have used microorganisms such as *Galactomyces geotrichum* [20], *Phanerochaete chrysosporium* [21] and *Enterobacter* [22] for the same purpose.

This study focused on isolating, characterizing, and identifying a new bacterial strain from industrial textile dyes effluents. The strain was genetically assessed by 16S rRNA analysis and software. Finally, the isolated bacteria were grown in a culture medium enriched with an azoic dye, and their decolorization capacity was evaluated.

MATERIALS AND METHODS

Culture medium

The mineral salts medium (MSM) consisted of the following components in (g/L): $Na_2HPO_4 \cdot 12H_2O$ (1.5), KH_2PO_4 (1.5), $(NH_4)_2SO_4$ (2.0), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.010), and $FeSO_4 \cdot 7H_2O$ (0.0010). The MSM was mixed with 0.010 g/L of the Golden Yellow K2R dye (Figure 1) and with agar (1.9% W/V) used to isolate and maintain a pure culture. The media was sterilized at 121 °C for 20 min before use [23].

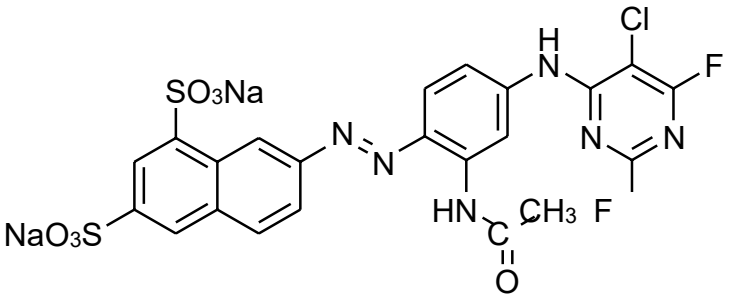

Figure 1: Chemical structure of the Golden Yellow K2R dye.

Obtaining the sample

A sample of textile effluent was taken to the laboratory to isolate the bacteria. 1.5 mL of effluent was placed in each of the 10 Eppendorf tubes before being taken to the microcentrifuge and run at 10,000 RPM for 5 min. The supernatant was eliminated, and the pellets formed in each tube were gathered. Subsequently, 100 µL of the sample was seeded using a Drigalsky loop on a plate with MSM agar and the Golden Yellow K2R dye as the only carbon source. The sample was incubated at 37°C for 24 hours.

Picking

A colony was removed from the initial seeding. It was placed in an Eppendorf tube with 1 mL of Triton and vortexed for 5 min. Then, it was seeded with a cole loop on a new plate. A total of 6 pickings were made following the same procedure.

Antibiogram

To discover the sensitivity of the isolated bacteria to different antibiotics, a sample (of the pure culture in MSM broth) was seeded on nutritive agar using a sterile swab. Then, paper discs with the chosen antibiotics for gram-harmful bacteria such as neomycin, cefazolin, ceftriaxone, ampicillin/sulbactam, cotrimoxazole, amikacin, lincomycin were placed on the agar. Following the Bauer-Kirby disc diffusion procedure, antibiotics were impregnated on absorbent paper discs [24]. The discs were distributed symmetrically over the surface of the culture medium inoculated with the bacterial strain. After incubation for 48 hours at 37°C, the inhibition halos produced by the antibiotics were measured.

DNA extraction

An Eppendorf tube was filled with 100 µL of microbeads and 500 µL of sterile water, and a loop of bacteria was dissolved. Then, it was vortexed at maximum speed for 5 min. After that, 500 µL of a phenol-chloroform-isoamyl alcohol mixture was added during the vortexing, and lastly, it was placed in the microcentrifuge at 15,000 rpm for 10 min. The supernatant was recovered by adding 600 µL of isopropyl alcohol, mixed gently and then centrifuged at 15,000 rpm for 10 min. After discarding the supernatant, ethanol was applied to dry

the pellet, and finally, it was re-suspended with 50 µL of sterile water, keeping the DNA at 4 °C. The technique used is a variant of the optimal technique for bacterial DNA isolation by bead beating described by Fujimoto *et al.* [25].

Sequencing the 16S rRNA gene of the 35-DOR_27F bacteria

Once the DNA extraction had been verified, the 16S rRNA gene was amplified by the PCR method. The obtained product was purified and sent for sequencing to the Instituto de Biotecnología de ADN de Uchumayo using the FinchTV software. Sequencing analysis of the 16S rRNA gene was performed at the Laboratory of Molecular Biology, Harvard University, Cambridge, MA, USA.

Bioinformatic analysis by NCBI BLAST

For the bioinformatic analysis, the NCBI BLAST database was used. The previously obtained DNA sequence was inserted. Likewise, in order to obtain the phylogenetic tree, the BioEdit, Mega and Trie View Explorer software were used. The 16S rRNA gene sequence of bacterial strain 35-DOR_27F was inserted into the NCBI GenBank database for alignment and comparison with stored bacterial sequences to assess their degree of homology using the BLASTn algorithm [26]. BioEdit, Mega and Trie View Explorer software were used to obtain the phylogenetic tree.

Biotechnological potential studies

100 µL of the isolated bacterial strain was inoculated in 10 mL MSM broth with Golden Yellow K2R dye (50 mg/L). Incubation was carried out at 37 °C for 72 hours. Afterward, the broth was centrifuged at 6000 rpm for 20 min. Then, the supernatant was separated, and its absorbance was measured at 388 nm (corresponding to the λmax of the dye) in a Thermogenesis UV–visible spectrophotometer. For the biodegradation study, an Agilent Technologies Cary 60 UV-V spectrophotometer was used to quantify the absorbance of a 60 mg/L solution of the yellow dye K2R. The isolated strain was cultured in peptonized broth for 48 h at 37°C, and then 1 mL of inoculum was taken and placed in a flask containing 100 mL of a 50 mg/L solution of the dye in peptonized water. The percentage of decolorization was evaluated after 72 hours using Equation 1:

$$\%Decolorization = \frac{Initial\ Abs - Final\ Abs}{Initial\ Abs} \times 100 \quad (1)$$

RESULTS

Morphological and cultural characteristics

Gram-negative bacteria were isolated with the following characteristics: circular point shape, whole border, flat colony elevation, creamy consistency, smooth surface and transparent white pigmentation.

Antibiogram

Results of the susceptibility of the isolated bacteria are shown in Table 1. After 24 hours of incubation, the bacteria were sensitive to most chosen antibiotics except ampicillin/sulbactam. Amikacin was the antibiotic that presented the largest halo, indicating that the isolated bacteria have a greater sensitivity to said antibiotic.

Antibiotics	Halo diameter (cm)	Sensitive/ Resistant
Neomycin	2.0	Sensitive
Cefazoline	1.5	Sensitive
Ceftriaxone	1.4	Sensitive
Ampicillin/Sulbactam	-	Resistant
Cotrimoxazole	2.8	Sensitive
Amikacin	3.0	Sensitive
Lincomycin	1.8	Sensitive

Table 1. Samples resistance to antibiotics.

Figure 2 shows the inhibition halos for the different antibiotics with the isolated bacteria on nutrient agar. The biggest inhibition halo corresponds to the antibiotic amikacin.

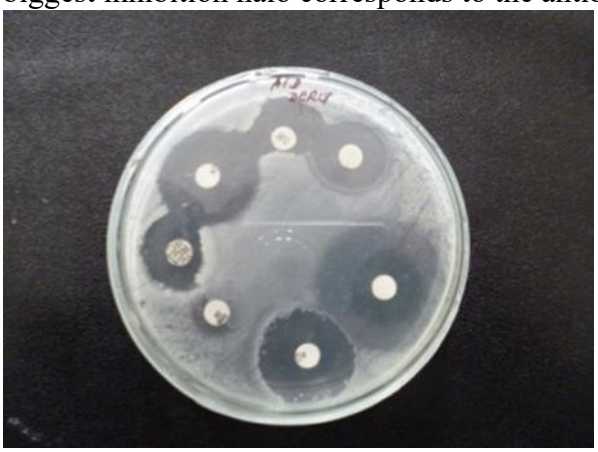

Figure 2: Antibiogram of the isolated bacteria.

The sequence of the 16S rRNA gene of the 35-DOR_27F bacteria

Using the FinchTV software, it was possible to observe the sequence of the public and pyrimidine bases of the extracted DNA, which is shown in Figure 3.

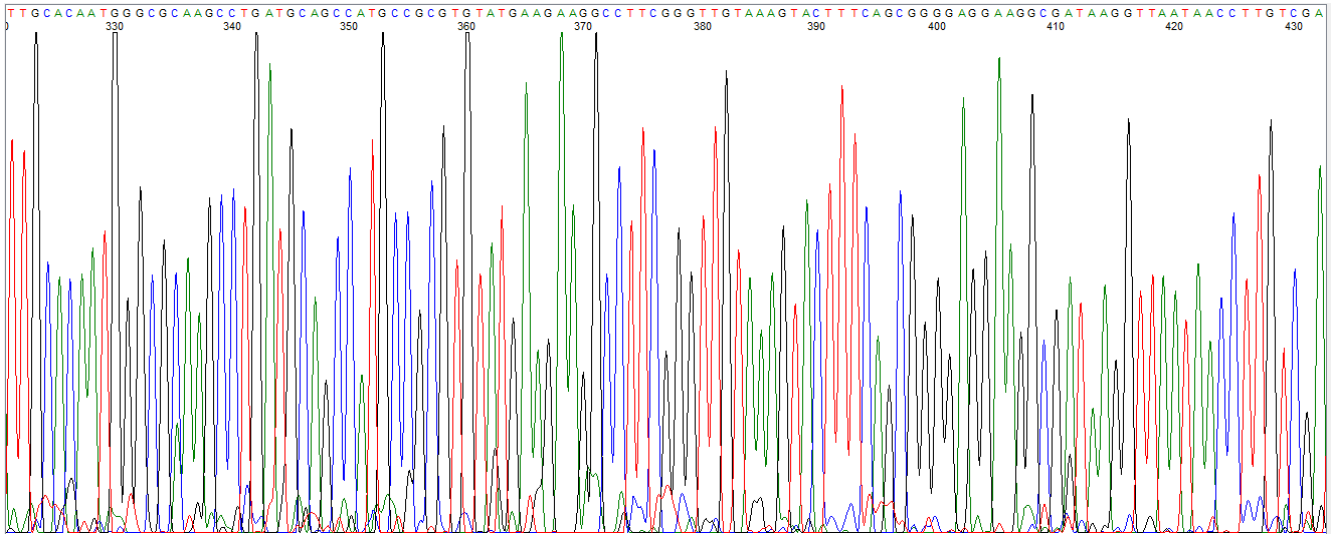

Figure 3: Visualization of the bacterial DNA sequence using FinchTV software.

Bioinformatic analysis using NCBI BLAST

After the sequence was obtained and the bioinformatic analysis using BLAST was performed, it was determined that the isolated strain shares a high percentage of similarity (99 %) with *Klebsiella* sp., which is cataloged as a species capable of decolorizing textile effluents. Figure 4 shows the results of the BLAST analysis with the greatest similarity to *Klebsiella* sp., and Figure 5 shows the phylogenetic tree; it is notable that the bacterium encoded as 35-DOR_27F is found at the same height as *Klebsiella* sp., which indicates it could belong to this species.

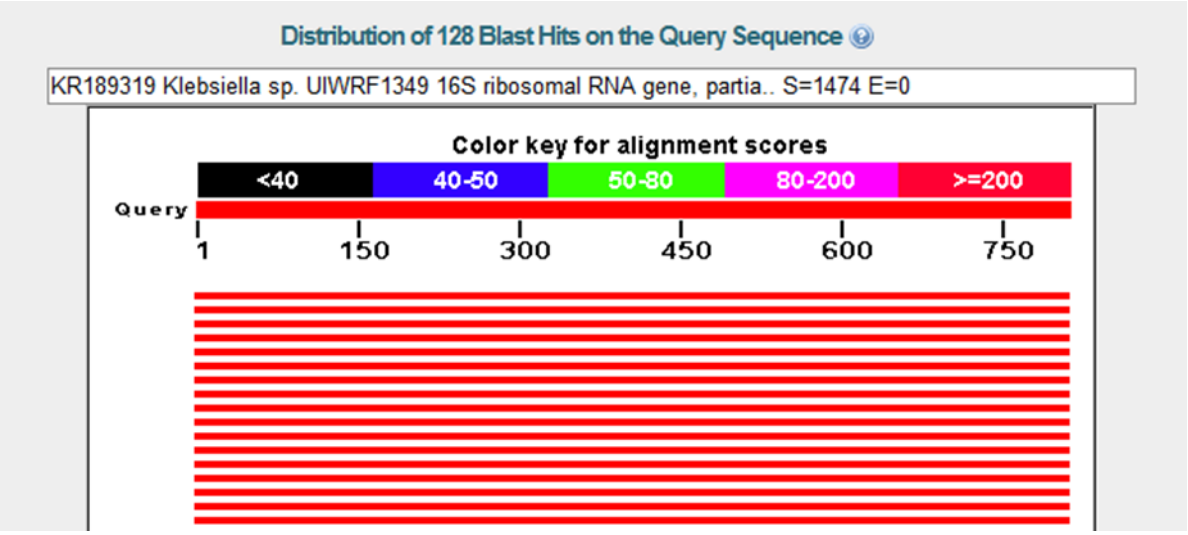

Figure 4: Analysis of the isolated bacteria using BLAST.

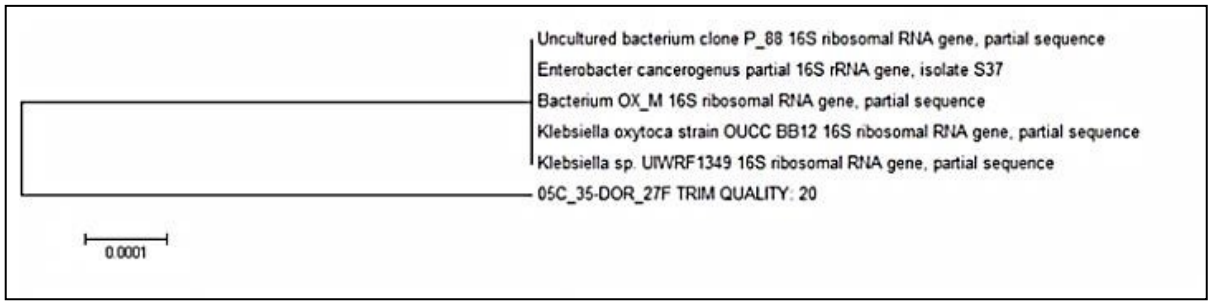

Figure 5: Phylogenetic tree of the isolated bacterium.

Biotechnological potential studies

The wavelength of maximum absorption for a 60 mg/L solution of Golden Yellow K2R was 388 nm with an absorbance of 0.9522. Figure 6 presents the spectrum obtained.

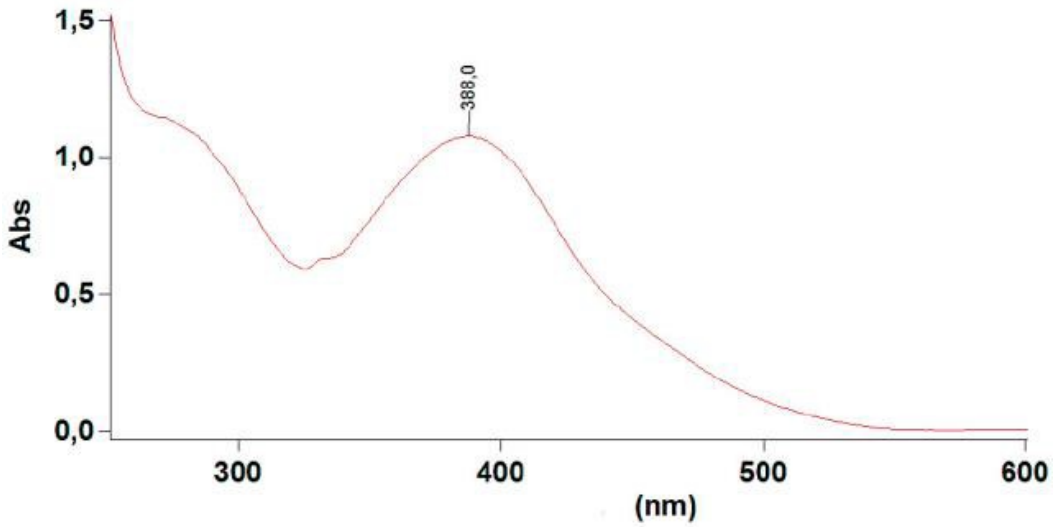

Figure 6: Spectral scanning of the Golden Yellow K2R dye.

After 72 hours of incubation, the results indicated that the decolorization of Golden Yellow K2R by the isolated strain identified *as Klebsiella* sp. was 74 %, suggesting that the isolated bacteria could be used in bioremediation processes.

DISCUSSION

Different bacterial species have been isolated from textile sources, demonstrating their bioremediation capacity against azoic dyes in solution. *Bacillus* and *Alcaligenes* stand out among the identified genera, as they are efficient when treating Novacron Super Black G dye 6. In addition, Alcaligenes aquatilis proved effective in degrading Synazol Red 6HBN colorant (82 %), suggesting it may be helpful to in wastewater treatment 27. Furthermore, *Bacillus subtilis*, *Bacillus megaterium*, *Erysipelothrix* and *Amphibacillus xylanus* were isolated and identified, and their degradation ability was studied against 7 azoic dyes. Results demonstrated that the dyes were decolorized (at least 50 %) after 3 days of incubation. However, none of the isolates could decolorize Novacron Red 28. Moreover, *Alishewanella* sp. has also shown decolorization capacity against Sumifex Tourqi blue (83 %) after 6 days, thus showing its potential for removing azoic dyes 29.
In the present research study, the isolated bacterial strain corresponds to *Klebsiella* sp., which was grown on MSM broth enriched with Golden Yellow K2R azoic dye and showed a decolorization of 74 %. Other studies report bacterial growth in agar enriched with different azoic dyes, such as Bezema Yellow S8-G and Bezema Red S2-B, showing decolorization rates of up to 90 % 8.

CONCLUSIONS

A new bacterium obtained from textile effluents was isolated, and the code 35-DOR_27F was assigned. Sequencing the 16S rRNA gene was found to correspond to *Klebsiella* genus. Furthermore, after 72 hours of incubation, it exhibited a decolorization percentage of 74 % of the Golden Yellow K2R dye. This
the study indicates that this bacterium has promising characteristics for addressing pollution caused by textile effluents.

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Conflicts of Interest: The authors declare no conflict of interest.

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