ISOLATION AND IDENTIFICATION OF NOVEL BACTERIAL STRAINS WITH CRYSTAL VIOLET BIODEGRADATION POTENTIAL FROM TEXTILE EFFLUENT WASTEWATER IN EGYPT

Yomna A. Hashem[1*], Dina Osama[2]

[1]Department of Microbiology, Faculty of Pharmacy, The British University in Egypt, Cairo, Egypt

[2]Department of Microbiology and Immunology, Faculty of Pharmacy, October University for Modern Sciences and Arts, Cairo, Egypt

Corresponding author: E-mail: Yomna.hashem@bue.edu.eg

Abstract

The textile industry is a significant player in the global economy; however, it is known for its contribution to environmental pollution by discharging untreated wastewater containing synthetic dyes. Crystal violet is a commonly used dye in the textile industry but has a harmful effect on humans and the environment. Therefore, developing a sustainable, environmentally friendly, and cheap method for crystal violet biodegradation like microbial biodegradation is crucial. To isolate bacteria degrading crystal violet, textile effluent wastewater was collected from different dying plants in Egypt. The degradation capacity of the bacteria isolated from textile effluent was measured using a spectrophotometer. The two bacterial isolates showing maximum crystal violet degradation FSL-1 and EBA-2, were identified using 16S rRNA as *Klebsiella pneumoniae* and *Pseudomonas oleovorans,* respectively. The effect of different parameters, including incubation time, initial dye concentration, pH, temperature, carbon source, agitation, and media composition, was studied on the ability of the two isolates to degrade crystal violet. The highest degradation of 92% was observed with *K. pneumoniae* isolate when inoculated in MSM-Y media containing 0.01 g/L crystal violet when incubated at 37 °C for 48 h under agitated conditions and pH 7; however, complete crystal violet degradation was observed when MSM-Y was substituted with brain heart infusion broth under the same conditions. Therefore, the newly identified bacterial strains in this study can be effectively utilized for the treatment of textile effluent containing crystal violet when certain parameters are optimized.

Keywords: crystal violet, biodegradation, textile effluent, *Pseudomonas*, *Klebsiella*

Introduction

Nowadays, the textile industry's growth is unprecedented and plays a significant role in the global economy. However, it is associated with environmental pollution (Li *et al*., 2023). The textile industry produces large amounts of wastewater that contains a high number of contaminants; one of the significant wastewater contaminants is textile dyes (Al-Tohamy *et al*., 2022).

Around 700,000 tons of dyes are consumed annually in the textile industry, and the unfixed dyes to the fabrics represent 10 to 15%, eventually discharged as effluent and wastewater into the environment (Natarajan & Manivasagan, 2013). Unlike natural dyes, synthetic dyes have a complex structure, making them toxic and resistant to degradation (Rapo & Tonk, 2021). Synthetic dyes in the water interfere with the penetration of sunlight, affecting the photosynthesis process and reducing the amount of dissolved oxygen in the water (Jamee & Siddique, 2019).

Crystal violet is a synthetic dye used in the textile industry for dying wool, silk, and cotton and is also used in medicine as a biological stain (Al-Tohamy *et al*., 2022; Maniyam *et al*., 2022; Varjani *et al*., 2020). Crystal violet has been labeled as a recalcitrant molecule, implying that microbes poorly metabolize it and, as a result, have a long half-life in various environments (Xiao *et al*., 2020). Studies on crystal violet have revealed that it is a mitotic poison, a potent carcinogen, and can promote tumor growth in some fish species (Mani & Bharagava, 2016).

Chemical methods like coagulation and ozonation and physical methods like adsorption and filtration are employed to treat wastewater containing dyes (Al-Tohamy *et al*., 2022; Varjani *et al*., 2020). Most chemical and physical processes are expensive and generate toxic byproducts. On the other hand, the biological method is inexpensive, environmentally friendly, and does not generate a large amount of toxic byproducts (Al-Tohamy *et al*., 2022; Natarajan & Manivasagan, 2013).

The current study aimed to isolate and characterize bacteria degrading crystal violet from textile industry effluents and identify the parameters that maximize the degradation capacity of these isolates.

Materials and Methods:

Chemicals and dyes

Crystal violet was purchased from Oxford, UK. The components of MSM media, including $MgSO_4$, $CaCl_2$, NH_4Cl, K_2HPO_4, NaCl, $MnSO_4$, $ZnCl_2$, $CuSO_4$, $FeSO_4$, glucose, and yeast extract were purchased from Sigma Aldrich, USA. HCl and NaOH were purchased from El Gomhoreya Co., Egypt.

Sample collection

The textile effluent wastewater samples were obtained from the static textile effluents of four local textile dyeing plants' drainage canals in Giza, Obour, and Zagazig cities in

Egypt. The wastewater samples were collected in sterile containers, transferred to the lab as soon as possible, and kept in the refrigerator at 4 °C for later processing.

Bacterial isolation

The enrichment culture technique modified by Roy *et al.* (Roy *et al*., 2018) was utilized to isolate bacteria degrading crystal violet from the textile effluent wastewater. Samples were diluted by transferring 1 ml of each sample to 9 ml of sterilized distilled water. Diluted samples were vortexed, and 1 ml of each sample was added to 9 ml of the enrichment media and incubated at 37 °C for 24 h. The enrichment medium in the current study was mineral salt medium (MSM) supplemented with 1 g/L yeast extract (MSM-Y) and 0.1 g/L crystal violet (Kamal *et al*., 2022). The MSM was prepared according to Hashem *et al*., with modifications. Briefly, 0.1 g $MgSO_4$, 0.2 g $CaCl_2$, 0.6 g NH_4Cl, 1.36 g K_2HPO_4, 0.5 g NaCl, 0.001 g $MnSO_4$, 0.002 g $ZnCl_2$, 0.002 g $CuSO_4$, 0.001 g $FeSO_4$, and 0.01 g glucose were dissolved in 1 liter of distilled water and sterilized (Hashem *et al*., 2018). After incubation, samples showing visible degradation **(Fig. 1)** were streaked on MSM-Y agar containing 0.1 g/L crystal violet and incubated at 37 °C for 24 h. Colonies showing clear degradation zones around them **(Fig. 2)** were picked and inoculated separately in MSM-Y broth with 0.1 g/L crystal violet and incubated at 37 °C for 24 hours. After incubation, 1 ml of the broth was withdrawn aseptically and centrifuged at 10,000 rpm for 15 minutes. The supernatant absorbance was measured and compared to a control containing broth and crystal violet using a spectrophotometer at 600 nm. This step determined the isolates with the maximum capacity to degrade crystal violet. Isolates with maximum degradation capacity were selected for further processing and preserved as glycerol stocks at −80 °C freezer.

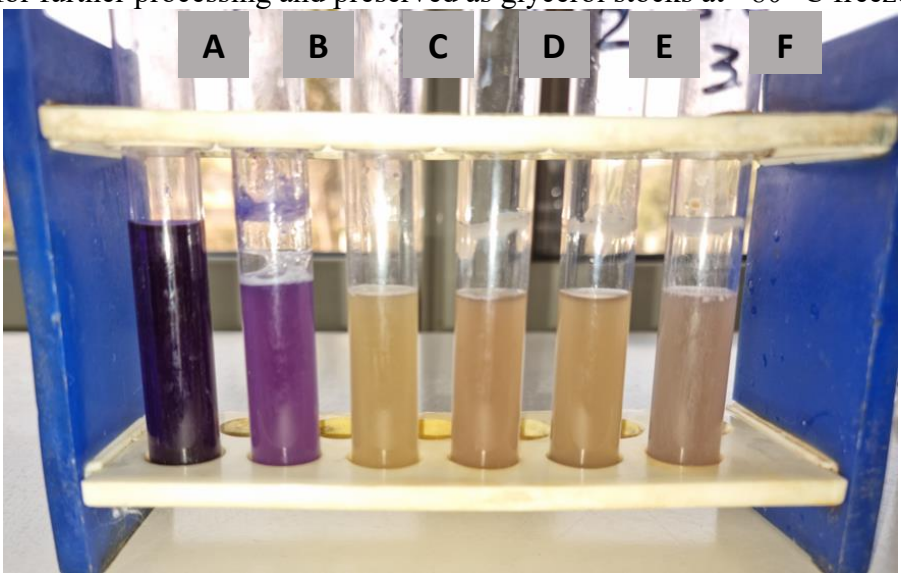

Figure 1. Biodegradation of crystal violet in mineral salt media with yeast (MSM-Y) broth containing crystal violet **(A)** control of MSM-Y broth and crystal violet only, **(B)** MSM-Y broth containing crystal violet and isolate showing partial degradation of crystal violet, **(C-F)** MSM-Y broth containing crystal violet and isolates showing complete degradation of crystal violet

Figure 2. Colonies showing clear degradation zones on MSM-Y agar containing crystal violet

Identification of selected bacterial isolates.

Isolates degrading crystal violet were preliminarily identified by Gram staining and biochemical tests like catalase, oxidase, indole, citrate, and triple sugar iron tests.

Identification of isolates degrading crystal violet by 16S rRNA sequencing

The molecular identification of the isolates showing the maximum degradation activity was done by sequencing the 16S rRNA gene. The DNA of the isolates degrading crystal violet was extracted from an overnight pure bacterial culture using a QIAamp DNA mini-kit (Qiagen, USA) as per the manufacturer's instructions. The total DNA concentration and purity were determined using a nanodrop spectrophotometer V-630 (Unicam, UK).

The 16S rRNA genes of the isolates degrading crystal violet were amplified by PCR using universal primers 27 F 5′AGAGTTTGATCMTGGCTCAG-3′ and 1492 R 5′-CGGTTACCTTGTTACGACTT-3′. The PCR amplification was performed in a SensoQuest (Germany) thermocycler, and the reaction mixture of a total of 20 µl consisted of 0.2 µl Taq polymerase (2.5 U/ µl) (Promega, USA), 2 µl buffer (10×), 1.6 µl 2.5 mM dNTP mixture, 1 µl (10 pmol/ µl) of each primer used, 1 µl DNA template (20 ng/ µl) and nuclease-free water up to 20 µl. Amplification was done by initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec., annealing at 55 °C for 1 min., extension at 68 °C for 1 min., and final extension at 68 °C for 10 min. PCR-amplified products were run using gel electrophoresis and purified using the Montage PCR Clean-up kit from Millipore (Merck, Germany) according to the manufacturer's instructions. The purified PCR products were sequenced using the Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA).

Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA) at Macrogen, Inc., Seoul, Korea. The generated sequences were assembled and analyzed using SeqMan Pro v10.0.1 (DNASTAR, Madison, WI, USA). The obtained sequences were submitted to the NCBI GenBank database, and sequence alignment was carried out using the online BLASTN tool to identify the best matching sequence and identify the microorganisms (Altschul *et al*., 1990).

Measurement of crystal violet degradation:

The degradation activity of the isolates was determined by monitoring the reduction of the absorbance at the absorption maxima (λmax) of crystal violet. The overnight cultures were inoculated in MSM-Y broth containing 0.01 g/L crystal violet and incubated at 37 °C for 24 h. The experiment was carried out in triplicate, and uninoculated MSM-Y containing 0.01 g/L crystal violet was used as a reference. At time intervals, 1 ml of the sample was aseptically withdrawn and centrifuged at 10,000 rpm for 20 min to separate the bacterial cells, supernatant was used to measure the concentration of the remaining crystal violet. The concentration of crystal violet was determined by measuring the absorbance of the supernatant at 600 nm using a spectrophotometer (Unicam, UK). Degradation activity was calculated according to the equation (Chen *et al*., 2003).

$$\text{Degradation (\%)} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

Effects of different physicochemical parameters on crystal violet degradation

The best two isolates showing maximum degradation activity were selected for further processing.

Effect of incubation time

To study the effect of incubation time on degradation, 1 ml from an overnight culture of the tested isolates was adjusted to 0.5 McFarland and inoculated in 9 ml of MSM-Y broth containing 0.01 g/L crystal violet and incubated at 37 °C. The degradation activity was measured at 24 and 48 hours.

Effect of initial dye concentration

To determine the effect of the initial dye concentration on the ability of the isolates to degrade crystal violet, 1 ml from an overnight culture of the tested isolates was adjusted to 0.5 McFarland and inoculated into 9 ml of MSM-Y broth containing crystal violet with concentrations ranging from 0.01 g/L to 0.125 g/L, and the tubes were incubated under static conditions at 37 °C. The degradation activity was measured after 48 h.

Effect of pH

To study the effectiveness of the isolates in degrading crystal violet at different pH values, 1 ml from an overnight culture of the tested isolates was adjusted to 0.5 McFarland and inoculated into tubes containing 9 ml of MSM-Y broth containing 0.01 g/L crystal violet with pH range from 5 to 9. The pH was adjusted by 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. The tubes were incubated under static conditions at 37 °C, and the degradation was measured after 48 h.

Effect of temperature

To study the effect of incubation temperature on the isolates' degradation capacity, 1 ml from an overnight culture of the tested isolates was adjusted to 0.5 McFarland and inoculated in tubes containing 9 ml of MSM-Y containing 0.01/L of crystal violet and the isolates were incubated at temperatures of 30 °C, 37 °C, 45 °C, and 60 °C and the degradation was measured after 48 h.

Effect of carbon source

Few microorganisms can use dyes as a sole source of carbon; studies have reported that glucose is the best carbon source to maximize degradation (Das & Mishra, 2017). To evaluate the ability of the isolates to use crystal violets as the only carbon source, 1 ml from an overnight culture of the tested isolates was adjusted to 0.5 McFarland and inoculated into MSM-Y containing 0.01 g/L of crystal violet without the addition of glucose. The isolates were incubated under static conditions at 37 °C, and the degradation activity was measured after 48 h.

Effect of Agitation

To study the effect of the agitation and static conditions on degradation, 1 ml from an overnight culture of the tested isolates was adjusted to 0.5 McFarland and inoculated into tubes containing 9 ml of MSM-Y containing 0.01 g/L crystal violet, the tubes were incubated at static and agitated condition at 180 rpm at 37 °C. The degradation was measured after 48 h.

Effect of media composition

To determine the effect of media composition on the isolates' degradation capacity, 1 ml from an overnight culture of the tested isolates was adjusted to 0.5 McFarland and inoculated into MSM-Y containing 0.01 g/L of crystal violet and brain heart infusion broth containing 0.01 g/L of crystal violet separately. Incubation was carried out at 37 °C, and the degradation was measured after 48 h.

All the experiments were done in triplicate, and the degradation ability of the dye was measured in terms of percentage degradation.

Results

Isolation and identification of crystal violet degrading bacteria

The cultivation of the textile effluent wastewater collected from different cities in Egypt in MSM-Y containing crystal violet revealed that three isolates named FSL-1, EBA-2, and EBA-3 could degrade crystal violet with percentages of 80%, 68%, and 25%. FSL-1 and EBA-2 were selected for further processing. The preliminary identification of the two isolates using different biochemical tests identified FSL-1 as *Klebsiella* spp. and EBA-2 as *Pseudomonas* spp.

The identification of FSL-1 and EBA-2 to species level by 16S rRNA gene sequencing using BLASTN tool available at GenBank database showed that FSL-1, the closest hit was *K. pneumoniae,* with an identity of 99%. Whereas the closest hit for EBA-2 was *P. oleovorans,* with an identity of 98.56%.

The 16S rRNA sequence of FSl-1 and EBA-2 was submitted to the NCBI GenBank database with accession numbers of OR287479 and OR287502 respectively.

Effects of different physicochemical parameters on crystal violet degradation
Effect of incubation time on dye degradation

The degradation percentage of FSL-1 and EBA-2 after 24 hours of incubation was 80% and 68%, respectively. However, after 48 hours of incubation, the degradation percentage increased to 89% and 85% for FSL-1 and EBA-2, respectively **(Fig. 3).**

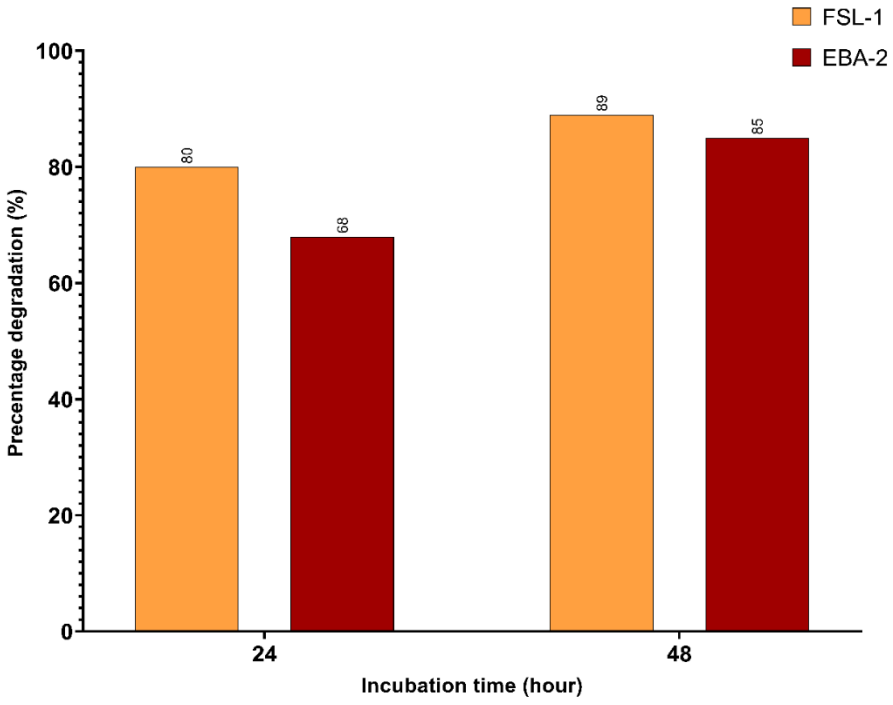

Figure 3 Effect of incubation time on crystal violet biodegradation

Effect of initial dye concentration on dye degradation

The isolates were assessed for their degradation activity using crystal violet concentrations ranging from 0.01 g/L to 0.125 g/L. After 48 hours of incubation, it was observed that FSL-1 achieved the maximum degradation of 89% at a concentration of 0.01 g/L; increasing the crystal violet concentration did not affect the ability of FSL-1 to degrade crystal violet. On the other hand, EBA-2 achieved maximum degradation of 85% at a concentration of 0.01 g/L; increasing crystal violet concentration decreased the degradation ability of EBA-2; at a concentration of 0.05 g/L and 0.075, the degradation decreased to 78%, and 72% respectively; and at a concentration of 0.1 g/L, the degradation was completely inhibited **(Fig. 4).**

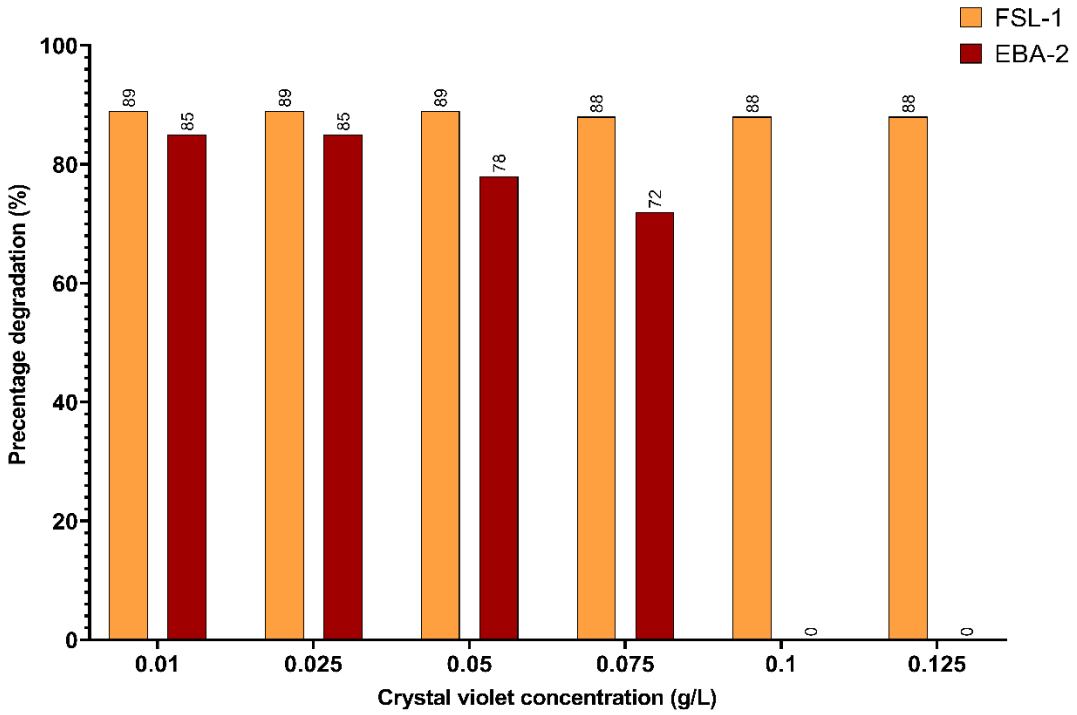

Figure 4 Effect of dye concentration on crystal violet biodegradation

Effect of pH on dye degradation

The crystal violet degradation efficiency of FSL-1 remained constant at approximately 88% ± 1 over the pH range of 5 to 9. In contrast, the degradation capacity of the EBA-2 isolate was 42% and 54% at acidic pH levels of 5 and 6, respectively. As the pH increased to 7, the degradation of EBA-2 reached 85% and maintained this level at pH values of 8 and 9 **(Fig. 5).**

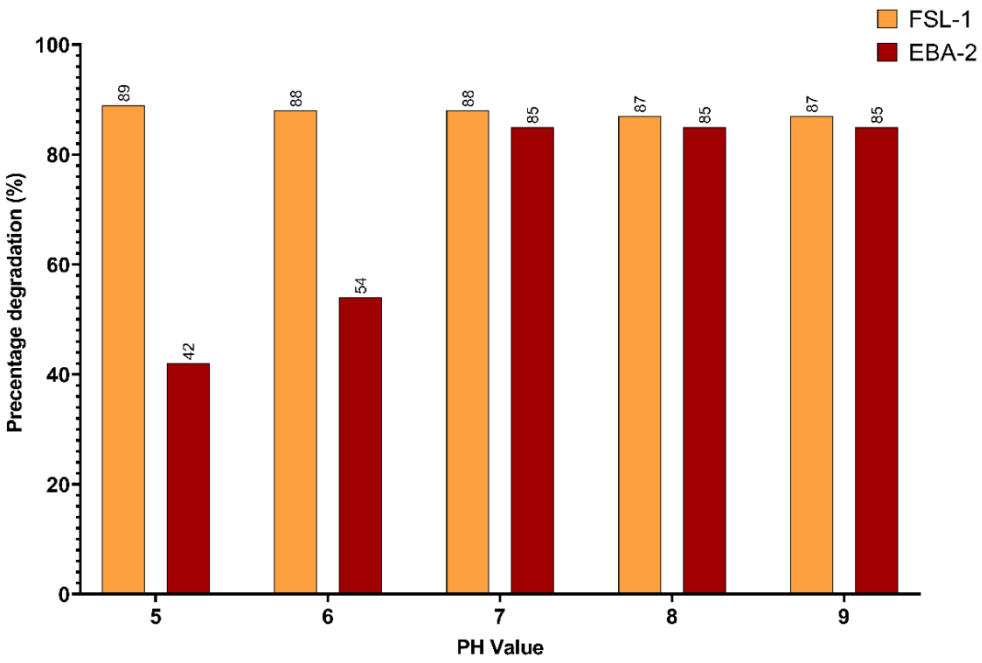

Figure 5 Effect of pH value of crystal violet biodegradation

Effect of temperature on dye degradation

The crystal violet biodegradation capacity of FSL-1 and EBA-2 remained almost unaffected within the temperature range of 30 °C to 45 °C. When the temperature increased to 60 °C, the degradation capacity of FSL-1 was completely inhibited, while EBA-2 exhibited a 33% degradation of crystal violet **(Fig. 6).**

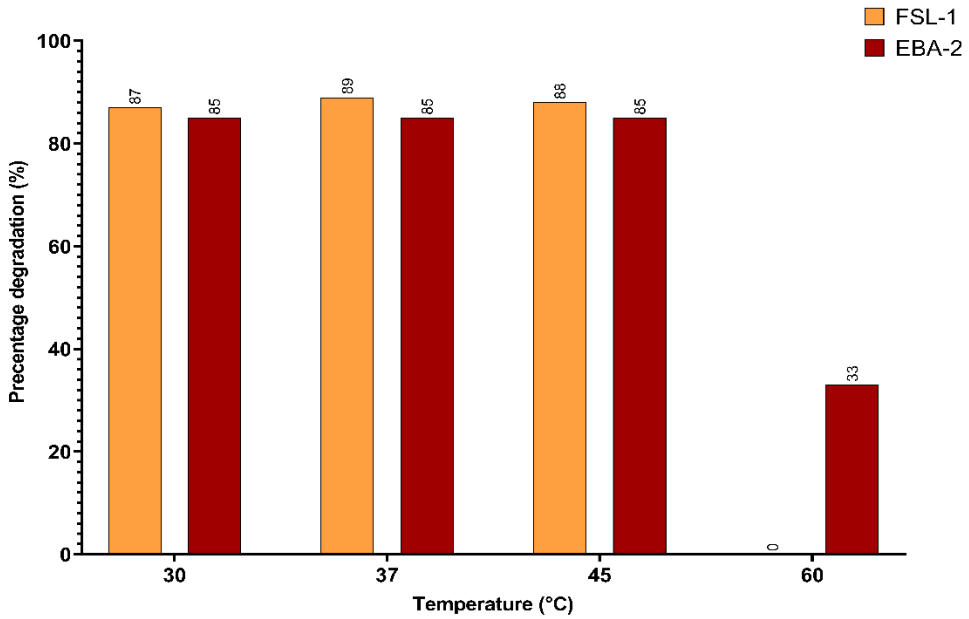

Figure 6 Effect of temperature on crystal violet biodegradation

Effect of carbon source

Upon the removal of glucose from the MSM-Y media, FSL-1 and EBA-2 demonstrated a lack of crystal violet degradation activity after 48 h of incubation.

The capacity for dye degradation under static and agitated incubation conditions

The agitation condition did not affect the crystal violet biodegradation capacity of FSL-1 after 48 h of incubation. However, the degradation efficiency of EBA-2 increased from 85% to 92% when incubated under static conditions for 48 h **(Fig. 7).**

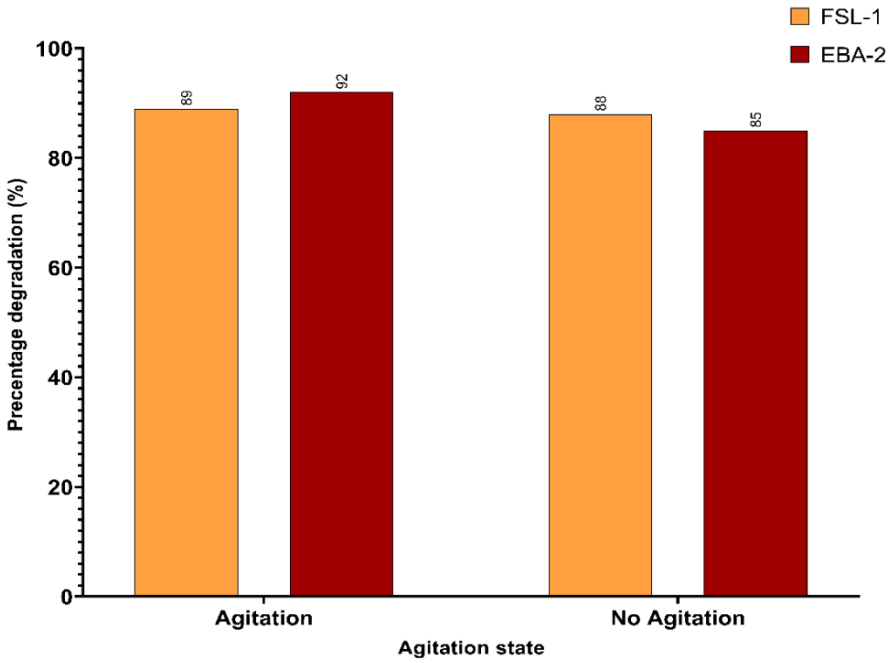

Figure 7 Effect of agitation on crystal violet biodegradation

Effect of media composition

The cultivation of FSL-1 in brain heart infusion broth achieved complete crystal violet degradation compared to 89% degradation in MSM-Y. It was also noted that degradation efficiency increased from 85% to 96% when EBA-2 was cultivated in brain heart infusion broth instead of MSM-Y **(Fig. 8).**

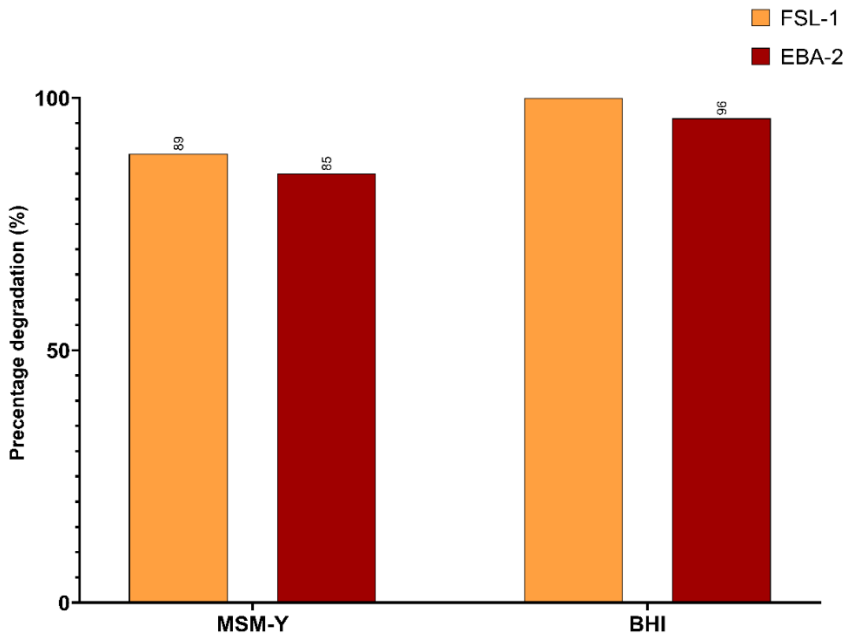

Figure 8 Effect of media composition on crystal violet biodegradation

Discussion

Crystal violet is one of the triphenylmethane dyes that is used in various industries such as textile, cosmetic, printing, pharmaceutical, and food processing (Ali *et al*., 2016; Mani & Bharagava, 2016); however, crystal violet is toxic and harmful to the environment and human health. Therefore, it is essential to remove it from wastewater before discharging it back into the environment. Biodegradation is one of the most effective and eco-friendly methods for removing crystal violet from wastewater. Several microorganisms have been identified as capable of degrading crystal violet (Azmi *et al*., 1998); however, some of these organisms were capable of degrading crystal violet at very low concentrations (Chen *et al*., 2003). Environmental parameters also play a key role in the ability of microorganisms to degrade crystal violet, so finding new microbial strains with the capacity to degrade higher concentrations of crystal violet with optimized parameters is important.

In our study, two bacterial strains isolated from textile effluent wastewater and identified as *K. pneumoniae* and *P. oleovorans* demonstrated the capacity for crystal violet degradation. Other studies identified other bacteria with crystal violet biodegradation capacity, including *Enterobacter* sp., *Pseudomonas putida*, and *Bacillus* sp. (Ayed*, , et al., 2009; Chen et al., 2003; Roy et al*., 2018). In this study, the two bacterial isolates showed a proportional increase in the degradation of crystal violet as the incubation time for both isolates increased from 24 to 48 hours. This finding was consistent with findings from earlier studies that demonstrated similar outcomes (Afrin *et al*., 2021; Khan *et al*., 2021; Khan & Joshi, 2019).

In addition to incubation time, other parameters, including the type of bacteria, the initial dye concentration, temperature, and pH, influence the biodegradation of

crystal violet (Ayed, *et al*., 2009). The increase in the crystal violet concentration from 0.01 g/L to 0.125 g/L did not affect the degradation capacity of *K. pneumoniae* isolate. However, in the case of *P. oleovorans* isolate, increasing the concentration of crystal violet led to a decrease in the degradation capacity. Other studies found that increasing the initial dye concentration led to a decrease in biodegradation efficiency (Ayed, *et al.*, 2009; Roy *et al*., 2018). The decrease in biodegradation can be attributed to a reduction in the ratio of nucleic acid content, leading to a decrease in protein synthesis that hinders cell division and the toxic effect of dyes (Khehra *et al*., 2006; Roy *et al*., 2018).

K. pneumoniae isolates also revealed almost constant degradation efficiency over the pH range of 5 to 9. On the other hand, the degradation capacity of *P. oleovorans* isolate was shifted towards higher pH; these findings are consistent with Roy *et al*. and Wang *et al.* who found that the maximum degradation of crystal violet by *Enterobacter* and reactive red 180 by *Citrobacter* occurs at pH 6.5 to 7 (Roy *et al*., 2018 ; Wang *et al*., 2009). In another study, the maximum efficiency in biodegradation of azo dye by *Aspergillus niger* occurred at pH 9 (Mahmoud *et al*., 2017). Therefore, the impact of different pH ranges on bacterial biodegradation is dependent on the bacterial strain and the degradation process conditions, which could be beneficial for bioprocessing dye-containing wastewater and useful for industrial applications (Li *et al*., 2014). Since textile industrial processes are typically carried out under alkaline conditions, having a high tolerance to high pH levels is important. The optimal pH range for the degradation process is often between 6.0 and 10.0 (Saratale *et al*., 2011).

Temperature is one of the most challenging environmental factors that affect dye degradation. It has an impact on bacterial growth and metabolic activities, including biodegradation, which is dependent on the performance of enzymes that are sensitive to temperature (Shi *et al*., 2021). In the current study, *K. pneumoniae* was able to degrade crystal violet dye at temperatures between 30-45°C. *P. oleovorans* demonstrated good activity at the same temperature range; moreover, it could degrade crystal violet by 33% at 60°C. The optimal range of 30°C to 40°C for the biodegradation of dyes has also been proven in other studies (Kochher & Kumar, 2011; Perumal *et al*., 2012; Roy *et al*., 2018).

The presence of additional carbon or nitrogen sources in the medium can enhance the degradation process by supporting bacterial growth (Jamee & Siddique, 2019; Kumar & Mongolla, 2015; Shi *et al*., 2021). However, some studies have reported that bacteria can use the dye as a sole carbon source**,** leading to successful dye degradation (Saratale *et al*., 2011). In our study, we found that the two isolates could not degrade crystal violet upon removal of glucose from the medium.

Several studies have reported that the efficiency of dye degradation by microorganisms is greatly impacted by agitation compared to incubation in static conditions *(Kamal et al., 2022; Saratale et al*., 2011). In the current study, agitation did not affect the biodegradation capacity of *K. pneumoniae*; on the other hand, agitation improved the degradation efficiency of *P. oleovorans* and increased it from 85% to 92%. This finding is consistent with the work of Kumar, Roy, and their colleagues, who deduced that agitation could enhance the degradation of crystal violet by increasing the contact between bacterial cells and dye molecules (Kumar & Mongolla, 2015; Roy *et*

al., 2018). Improving the degradation by agitation can also be attributed to the enhanced activity of oxidative enzymes during aerobic conditions, which are increased by agitation (Khan *et al*., 2013).

The composition of the media can also play a role in the ability of the bacterial strains to degrade the dyes of interest (Al-Garni *et al*., 2013; Ghanem & Al-Garni, 2011). In our study, when mineral salt media was substituted with brain heart infusion broth, *K. pneumoniae* was capable of degrading crystal violet completely. For *P. oleovorans*, the degradation percentage increased from 85% to 96%.

Conclusion

In this study, two isolates identified as *K. pneumoniae* and *P. oleovorans* demonstrated significant potential for crystal violet biodegradation. *K. pneumoniae* achieved degradation of 88% when the concentration of crystal violet was increased up to 0.125 g/L in MSM-Y. The isolate showed stability over a wide range of pH and could degrade crystal violet at 45°C. When the isolate was cultivated in brain heart infusion broth, complete degradation of crystal violet was noticed. The *K. pneumoniae* strain identified in the current study can be employed in crystal violet biodegradation as it has shown high efficiency in biodegradation and consistency over the range of parameters evaluated.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Yomna A. Hashem designed the study and performed the practical work, Yomna A. Hashem and Dina Osama analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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عزل وتعريف سلالات بكتيرية جديدة ذات قدرة على التحلل الحيوي للصبغه البنفسجية البلورية من مياه صرف المنسوجات في مصر

يمنى عبدالجواد هاشم [١]*, دينا أسامة[٢]

[١] قسم الميكروبيولوجي، كلية الصيدلة، الجامعة البريطانية في مصر، القاهرة

قسم الميكروبيولوجيا و المناعة، كلية الصيدلة، جامعة أكتوبر للعلوم الحديثة و الآداب

البريد االلكتروني للباحث الرئيسي: yomna.hashem@bue.edu.eg

صناعة النسيج هي لاعب هام في الاقتصاد العالمي. ومع ذلك، تشتهر بمساهمتها في تلوث البيئة عبر تصريف المياه الصرف الغير معالجة التي تحتوي على أصباغ صناعية. الصبغه البنفسجية البلورية هي صبغة تستخدم على نطاق واسع في صناعة النسيج ولكن لها تأثير ضار على الإنسان والبيئة. لذا، يعتبر تطوير طرق مستدامة وصديقة للبيئة ورخيصة للتحلل البيولوجي للصبغه البنفسجية البلورية مثل استخدام البكتيريا ضروريًا. لفصل البكتيريا المحللة للصبغه البنفسجية البلورية تم جمع مياه الصرف الناتجة عن صناعة النسيج من مصانع مختلفة للصباغة في مصر. تم قياس قدرة تحلل الصبغه البنفسجية البلورية بواسطه البكتيريا المعزولة من مياه الصرف باستخدام مطياف الأشعة فوق البنفسجية. تم تحديد العزلتين اللاتي أظهرتا أقصى قدر من تحلل الصبغه البنفسجية البلورية وهما FSL-1 و EBA-2باستخدام 16S RNA كـ *Klebsiella pneumoniae* و*Pseudomonas oleovorans* . تم دراسة تأثير العوامل المختلفة على قدره البكتيرا على تحليل الصبغه، بما في ذلك وقت الحضانة والتركيز الأولي للصبغة ودرجة الحموضة ودرجة الحرارة ومصدر الكربون والتحريك وتكوين الوسط. تم رصد أعلى معدل تحلل بنسبة ٩٢% مع العزلةFSL-1 عندما تم زرعها في وسط MSM-Yيحتوي على ٠.٠١ g/L من الصبغه البنفسجية البلورية عند حضانة بدرجة حرارة ٣٧ درجة مئوية لمدة ٤٨ ساعة تحت ظروف تحريك ودرجة حموضة ٧. عند استبدال وسط ال MSM-Y بوسط Brain heart infusion broth لوحظ حدوث تحلل كامل للصبغه البنفسجية البلورية و ذالك تحت نفس الظروف. لذلك، يمكن استخدام السلالات البكتيرية المعرفة حديثًا في هذه الدراسة بفعالية لمعالجة مياه الصرف الناتجة عن صناعة النسيج التي تحتوي على الصبغه البنفسجية البلورية عند تحسين بعض العوامل.

الكلمات المفتاحية: الصبغه البنفسجية البلورية, التحلل الحيوي, مياه صرف المنسوجات, *Pseudomonas ,Klebsiella*