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Effect of UV mutation on phenol degraders obtained from petroleum areas of Churchgate

Powar Sakshi[1], Prabhudesai Shubham[2], and Kataria Roonal[*3]

[1]Department of Microbiology, Jai Hind College, Mumbai, powar.sakshi5172@gmail.com
[2] Department of microbiology, Jai Hind College, Mumbai, shubhamspd2002@gmail.com
[*3] Head, Department of Microbiology, Jai Hind College, Mumbai, rjroonal@gmail.com

Abstract: **Phenol has been widely used in the synthesis of organic chemicals and released into the aquatic environment through the effluent from coking plants, petroleum processing plants, chemical plants, pharmaceutical industries, etc. The elimination of phenol has drawn a lot of attention because it is harmful for aquatic ecosystems and pose a risk to people by contaminating drinking water sources. Phenol degraders are organisms that can utilize phenol as their carbon source, they take up phenol and convert it to catechol or protocatechuate by phenol hydroxylase enzymes which is further broken down. Genetic material can undergo mutations as a result of physical or chemical substances exposure. One of the physical methods for causing mutations by creating DNA cross-links and pyrimidine dimerization is UV exposure. Bacterial strains that degrade phenol were enriched and isolated from soil samples obtained from Bharat Petroleum corporation, Churchgate using phenol-containing Bushnell and Hass medium. The isolates were further exposed to UV light. The ultraviolet exposure improved the isolated organism's ability to tolerate phenol and degrade it at a higher concentration. A mutant strain with a greater phenol-degrading efficiency was chosen after being exposed to UV radiation for mutation for 120 seconds. Later, the degrading efficiency was calculated using the 4-aminoantipyrine technique. The results showed that there was an increase in the amount of tolerance and efficiency of degradation in the mutant strain.**

Index Terms: **4-Aminoantipyrine technique, Degradation efficiency, Mutant strain, Phenol, UV Exposure.**

I. Introduction

Phenol is a hazardous organic compound that is frequently used in industrial processes like coking, processing of petroleum, synthesis of organic chemicals, and the pharmaceutical industries. Due to the fact that these industries discharge phenol into the aquatic environment, which has the potential to seriously harm aquatic ecosystems and human health, phenol is a potential environmental pollutant. (Anku et al., 2017). The discharge of wastewater from numerous industries, including petroleum processing, chemical and coking plants, as well as pharmaceutical industries, is responsible for the release of phenol into the environment. (Villegas et al., 2016). The entire ecosystem can be impacted by higher levels of phenol leakage in the environment. Reactivity is demonstrated from the structure of Phenol, which causes it to be persistent in the environment and possibly carcinogenic to all living things. (Gami et al., 2014). As a result, removal of phenol from contaminated sites has received a lot of attention, and numerous techniques have been developed in order to tackle the mentioned problem. (Saputera et al., 2021). Due to the significance of phenol biodegradation in reducing the negative effects of phenol on the environment, it has been thoroughly studied. One of the most promising phenol removal techniques uses microorganisms that can break down phenol into less dangerous by-products. (Pradeep et al., 2015). Phenol degraders are microorganisms that can use phenol as a source of carbon. These organisms absorb phenol and use phenol hydroxylase enzymes to convert it to protocatechuate or catechol, which are then further broken down. (Nan et al., 2021). Exposure to chemical or physical substances can cause genetic material to mutate. Genetic material can be altered as a result of DNA cross-links and pyrimidine

* Corresponding author

dimerization brought on by UV radiation. (Cooper, 2000). Inducing genetic mutations through a physical process known as UV mutagenesis may improve microorganism's capacity to tolerate and break down phenol. In the last several years, mutational methods have been used to improve industrial strains, such as the improved biodegradation of a sulfonated azo dye, green HE4B in *Pseudomonas sp*. LBC1. In various studies, UV mutagenesis was used to increase the microorganisms' capacity to break down phenol. (Mao et al., 2015).

The objective of this study is to investigate the effect of UV mutagenesis on phenol biodegradation. Bacterial strains that degrade phenol were enriched and isolated from soil samples obtained from Bharat Petroleum corporation, Churchgate, using phenol-containing Bushnell and Hass medium. The isolates were further exposed to UV light, and the mutant strains were selected based on their ability to tolerate and degrade phenol at a higher concentration.

II. Materials and Methodology

A. Enrichment and isolation of phenol degraders

Bacterial strains that degrade phenol were isolated from soil samples obtained from Bharat Petroleum corporation, Churchgate, Mumbai, India. These soil samples were initially enriched using 50 ppm phenol-containing Bushnell and Hass broth. The samples were incubated at room temperature and kept on a rotary shaker for 2 days. Further, sequential enrichment of phenol degraders from soil samples was carried out using 100 ppm and 200 ppm phenol-containing Bushnell and Hass broth. Isolation of phenol degraders was carried out on Bushnell and Hass agar medium containing 200 ppm phenol. The plates were incubated at room temperature for 24-48 hours and the colonies that grew on the plates were selected for UV exposure (Patil et al., 2023). Gram staining of the obtained organisms was performed.

B. UV Mutagenesis

Two isolates namely C1 and C2 were inoculated in saline and their O.D. was adjusted to 0.1 at 530nm. 7 ml of culture suspension was transferred in petri plates and exposed to UV rays for 60 seconds and 120 seconds respectively using a Systronix UV chamber. Post mutation, 0.5 ml of the exposed culture was inoculated in 2 ml of Luria Bertani broth and incubated overnight. The UV-treated colonies obtained were termed C1-60, C1-120, C2-60 and C2-120. After 24 hours, the cultures were plated on Bushnell and Hass media containing 500ppm phenol (Jayanthi et al., 2015).

C. Determination of phenol tolerance

The phenol tolerance of the strains was determined using the broth dilution method. Tubes with varying concentrations of phenol ranging from 500-2000 ppm at intervals of 100 ppm were prepared and the isolates were tested for growth after 24 hours of incubation. MIC of phenol was determined based on the ability of the isolates to tolerate phenolic content (Jayanthi et al., 2015).

D. Efficiency of phenol degradation

The degrading efficiency of the mutant strains was determined using the 4-aminoantipyrine technique. In this technique, phenol reacts with 4-aminoantipyrine in the presence of an oxidizing agent to form a colored dye complex. The intensity of the color produced is directly proportional to the concentration of phenol present in the sample. This colored complex is measured spectrophotometrically at a wavelength of 540 nm, allowing for precise quantification. To determine the efficiency of phenol degradation, the six isolates were inoculated in B&H medium containing 1500 ppm phenol. Phenol estimation was carried out at intervals of 24 and 48 hours using the 4-aminoantipyrine method. Standard calibration curve was plotted using 10-50 ppm of known concentrations of phenol at intervals of 10 ppm. Percentage efficiency of phenol degradation of each isolates were calculated (Patil et al., 2023).

III. Results

A. Phenol degraders were isolated and gram-positive cocci and short rods were observed.

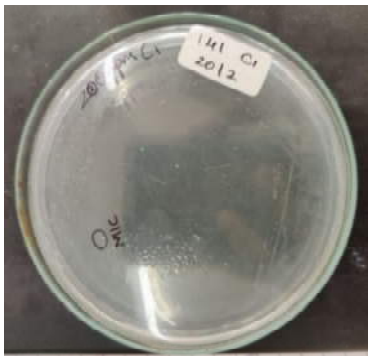

Fig. 1. Isolation of C1 on B&H containing 200ppm phenol

Fig. 2. Gram staining of isolate C1

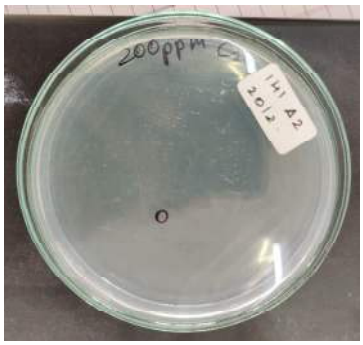

Fig. 3. Isolation of C2 on B&H containing 200ppm phenol

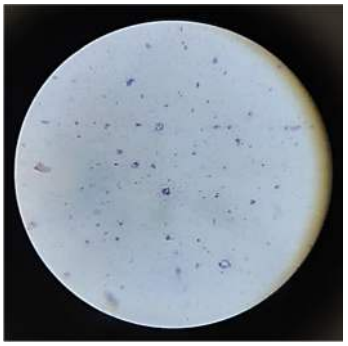

Fig. 4. Gram staining of isolate C2

Two bacterial isolates were recovered and tested for their ability to degrade phenol over a period of 24 and 48 hours. The results depict the isolation and characterization of phenol-degrading bacteria. “Fig. 1” and “Fig. 3” show the growth of bacterial isolates C1 and C2 on B&H agar containing 200 ppm phenol, indicating their ability to tolerate and potentially utilize phenol as a carbon source. The gram staining results, as seen in “Fig. 2” and “Fig. 4”, confirm that the isolates are gram-positive, with observations of cocci and short rod-shaped bacteria respectively.

B. Growth of mutants post UV mutagenesis

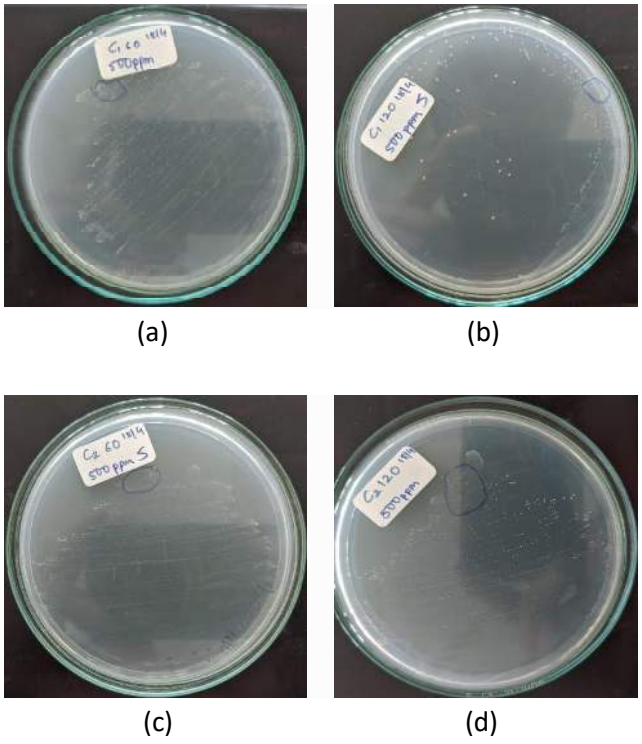

Fig. 5. Isolation of mutants (a) C1-60, (b) C1-120, (c) C2-60 and (d) C2-120 on B&H medium containing 500ppm

The results shown in the images depict the growth of bacterial mutants after UV mutagenesis on B&H medium containing phenol. “Fig. 5” presents the isolation of four different mutant strains: C1-60, C1-120, C2-60, and C2-120. The presence of bacterial colonies on the plates indicates that the mutants retained their ability to grow in the presence of phenol, potentially exhibiting enhanced tolerance or biodegradation efficiency.

C. Phenol tolerance of the mutated and unmated strains was obtained as follows-

Table I. Numerical values of phenol tolerance

Strain	Phenol tolerance (ppm)
C1	700
C2	600
C1-60	800
C1-120	1600
C2-60	900
C2-120	1200

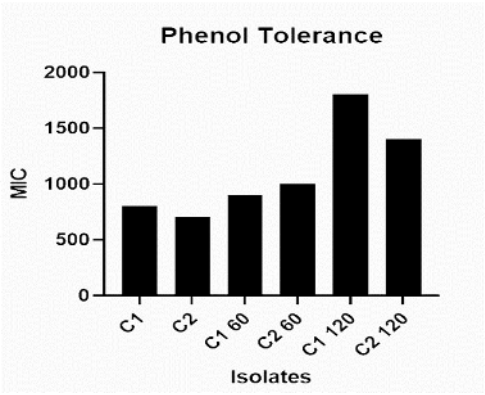

Fig. 6. Graphical representation of phenol tolerance

The results in “Table I” and “Fig. 6” illustrate the phenol tolerance levels of both mutated and non-mutated bacterial strains. “Table I” presents the numerical values of phenol tolerance in ppm, showing that the original isolates, C1 and C2, exhibited tolerance levels of 700 ppm and 600 ppm, respectively. However, after UV mutagenesis, the tolerance levels increased in all mutants, with C1-60 and C2-60 showing moderate improvements (800 ppm and 900 ppm, respectively), while C1-120 and C2-120 exhibited the highest tolerance (1600 ppm and 1200 ppm, respectively).

D. Efficiency of phenol degradation was determined using 4-aminoanityprene method

Table II. Remaining amount of phenol present (ppm) after 24 and 48 hours in the flask containing the respective organisms

Strain	24hr	48hr
C1-60	800	60
C1-120	1200	200
C2-60	1150	10
C2-120	900	40

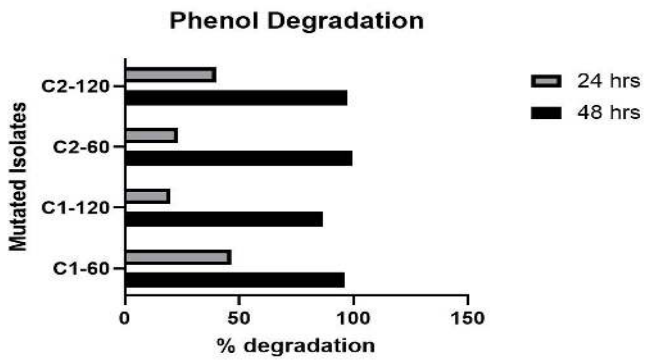

Fig. 7. Graphical representation of percent degradation of phenol by mutated and unmutated isolates in 24 and 48 hours

The results indicate that the degradation of phenol varies among different bacterial strains over 24 and 48 hours. The remaining phenol concentrations in the flask show that C1-60 and C2-120 exhibit the highest degradation efficiency initially, with C2-60 reducing phenol levels to just 10 ppm and C1-120 to 200 ppm after 48 hours. The graphical representation further confirms this trend, illustrating a significant increase in phenol degradation over time, particularly for C2-60 and C2-120 and C1-60. In contrast, C1-120 demonstrated lower degradation efficiency, retaining 200 ppm after 48 hours. These findings suggest that C2-60 is the most effective strain for phenol degradation, followed by C2-120 and C1-60, making them promising candidates for bioremediation applications.

IV. Discussion

The results of this study highlight the efficacy of UV mutagenesis in enhancing the phenol-tolerating capacity and biodegradation efficiency of microorganisms isolated from contaminated soil samples. Through UV exposure, the mutant strains exhibited notable improvements in their ability to tolerate and degrade phenol, which holds significant promise for environmental remediation efforts. UV mutagenesis has been a subject of interest in various biotechnological applications, particularly in enhancing the functional characteristics of microorganisms for environmental cleanup purposes. The findings presented here corroborate previous studies that have demonstrated the effectiveness of UV mutagenesis in inducing genetic alterations that can lead to enhanced phenol degradation capabilities (Mao et al., 2015)

The selection of mutant strains based on their ability to tolerate and degrade phenol at higher concentrations underscores the practical relevance of this research. The mutant strain C1-120, in particular, exhibited a remarkable increase in phenol tolerance compared to the wild type, demonstrating its capacity to tolerate up to 1600 mg/L of phenol within 48 hours. Such heightened tolerance levels are crucial for effective remediation of phenol-contaminated sites, where high concentrations of phenol pose significant challenges to microbial degradation processes.

Moreover, the observed increase in phenol degradation efficiency in the mutant strains further accentuates the potential of UV mutagenesis as a viable strategy for improving bioremediation efforts. The mutant strain C1-60 exhibited a 16.6% increase in degradation efficiency compared to the wild type, indicating the enhanced enzymatic activity or metabolic pathways involved in phenol degradation within the mutant strains.

The mechanisms underlying the enhanced phenol degradation efficiency in the mutant strains warrant further investigation. It is plausible that genetic alterations induced by UV mutagenesis have led to the upregulation of phenol hydroxylase enzymes or other phenol-degrading pathways, facilitating more efficient breakdown of phenol compounds (Heipieper et al., 1991). Additionally, changes in the cell membrane structure or the development of efflux pumps may contribute to the observed increase in phenol tolerance among the mutant strains. The potential applications of the mutant strains C2-60 and C2-120 in bioremediation efforts are promising. However, it is imperative to conduct further studies to evaluate the long-term stability and efficacy of these strains under diverse environmental conditions. Assessing the performance of the mutant strains in real-world scenarios, including variations in temperature, pH levels, and substrate concentrations, will provide valuable insights into their practical utility for phenol remediation purposes.

Furthermore, future research endeavors should explore the synergistic effects of combining the mutant strains C1-60, C2-60 and C1-120 in phenol degradation processes. Investigating the cooperative interactions between these strains may uncover novel strategies for optimizing phenol remediation efficiency and addressing challenges associated with environmental pollution.

In conclusion, this study underscores the potential of UV mutagenesis as a valuable tool for enhancing the phenol-tolerating capacity and biodegradation efficiency of microorganisms. The findings contribute to the ongoing efforts aimed at developing sustainable solutions for environmental remediation and underscore the importance of harnessing microbial diversity and genetic manipulation techniques in addressing contemporary environmental challenges.

Conclusion

The results show that UV exposure improved the isolated organism's ability to tolerate and degrade phenol at a higher concentration. A mutant strain with a greater phenol-tolerating capacity and degradation efficiency was chosen after being exposed to UV radiation for 120 and 60 seconds respectively. The mutant strain C1-120 had a greater tolerance for phenol than the wild type (700 mg/L), and it was able to tolerate 1600 mg/L of phenol in 48 hours. The degrading efficiency of the mutant

strain C1-60 increased by 16.6% compared to the wild type strain and it was able to degrade 600 ppm phenol in 24 hours. Their synergistic effect should be studied.

This study demonstrates that UV mutagenesis is an effective method for enhancing the tolerance and biodegradation efficiency of phenol-degrading microorganisms. The mutant strain's improved phenol degradation efficiency may be the result of genetic changes that raised the production or activity of phenol hydroxylase enzymes or other phenol-degrading enzymes. Changes in the cell membrane's structure or the development of efflux pumps that can expel phenol from the cell could be the reason for greater tolerance to phenol (Heipieper et al., 1991).

The mutant strains C1-60 and C1-120 obtained in this study can be considered as a potential option for bioremediation of phenol-contaminated sites. Based on morphology and Gram stain alone, and considering their ability to degrade phenol, the cocci could potentially be from the genus *Micrococcus*, known Gram-positive cocci, while the rods could possibly belong to the genus *Bacillus*, a well-known group of Gram-positive, rod-shaped bacteria. However, further studies are required to investigate the long-term stability and efficiency of the mutant strain under different environmental conditions. In order to assess the potential of this organism for its use in the bioremediation industry, we are presently identifying the organisms and further screening tests can be performed to check their rate of phenol degradation at varying concentrations, understanding organism's metabolic pathway, the effect of temperature and pH on tolerance and synergistic effect of both these mutant strains on degradation capacity.

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