Enhancement of Microbial Growth in *Pseudomonas* Species through Mutagenesis

Tosin Akin Akinmolayan [*1], Olutoyin Omolara Bamigboye [1], Jude Oluwapelumi Alao [*2], Eunice Damilola Wilkie [1], Favour Oluwadara Bamigboye [3], Oluwatosin Akinola Ajibade [*1]

[1] Microbiology Department, Adeleke University, Osun State, Nigeria
[2] School of Public Health and Interdisciplinary Studies, Auckland University of Technology, Auckland, New Zealand
[3] Department of Microbiology, University of Ibadan, Oyo State, Nigeria

Corresponding Authors: **Tosin Akin Akinmolayan** (tosin.akinmolayan@adelekeuniversity.edu.ng), **Jude Oluwapelumi Alao** (alao.jude@autuni.ac.nz), **Oluwatosin Akinola Ajibade** (oluwatosin.ajibade@adelekeuniversity.edu.ng)

Running Title
Mutagenesis-Enhanced Growth in *Pseudomonas*

Impact Statement

This study shows that ethidium bromide-induced mutagenesis can significantly enhance microbial growth in Pseudomonas species, particularly P. azotoformans and P. fluorescens, compared to wild-type strains. By optimizing bacterial isolates from maize cobs, the research presents a promising approach for improving the breakdown of lignocellulosic biomass. Although cellulase activity was not directly measured, the enhanced growth suggests potential for increased enzyme production. These findings offer a reproducible strategy for boosting microbial performance, with implications for biofuel development, environmental biotechnology, and industrial strain engineering.

Abstract

The production of cellulase enzymes is crucial for converting lignocellulosic biomass into fermentable sugars, a process essential for various industrial applications. This study investigates the impact of mutagenesis on microbial growth in *Pseudomonas* species, specifically *P. azotoformans*, *P. fluorescens*, and *P. lactis*, isolated from maize cobs. Mutagenic agents including ultraviolet (UV) radiation, ethidium bromide (EtBr), and nitrous acid (NA) were used to induce mutations, and bacterial growth was assessed over eight days using Congo Red carboxymethyl cellulose medium. While EtBr mutagenesis significantly enhanced bacterial growth, particularly in *P. azotoformans* and *P. fluorescens*, with growth increases of up to 73% at 192 hours compared to wild-type strains, cellulase activity was not directly measured in this study. UV and nitrous acid treatments showed only moderate or negative effects on growth. Although EtBr, a known carcinogen, raises safety concerns, its potential for improving microbial growth suggests it could be useful in optimizing strains for industrial applications. Further studies are needed to directly measure cellulase activity and confirm the impact of these mutagenic treatments on enzyme production.

Keywords: *Pseudomonas*; Mutagenesis; Ethidium Bromide; Nitrous Acid / UV Radiation; Microbial Growth; Lignocellulosic Biomass; Cellulase

Introduction

Cellulases are a group of complex enzymes predominantly produced by bacteria, fungi, and actinomycetes. They play a crucial role in hydrolysing cellulose into fermentable sugars, making them essential in a wide range of industrial applications, including biofuel production, paper and pulp processing, textiles, and the food and feed industries (Anoop Kumar et al., 2019; Chukwuma et al., 2020). The global industrial enzymes market, valued at $6.6 billion in 2021, is projected to reach $10.8 billion by 2029 (BCC Research, 2025), underscoring the growing importance of cellulases in industrial applications.

The conversion of lignocellulosic biomass into biofuels, particularly bioethanol, has gained significant attention as a sustainable alternative to fossil fuels (Jain & Kumar, 2024). Cellulases facilitate the breakdown of complex plant materials into simple sugars, which can then be fermented into bioethanol (Ranganathan et al., 2022). This process not only offers a renewable energy source but also plays a role in reducing greenhouse gas emissions. However, the recalcitrant nature of lignocellulosic biomass presents challenges, highlighting the need for efficient microbial growth and cellulase production to make biofuel production cost-effective.

Among microbial cellulases, those derived from bacteria, particularly *Pseudomonas* species, have emerged as promising candidates for industrial applications due to their robust enzymatic profiles. *Pseudomonas fluorescens* and *Pseudomonas lactis*, in particular, exhibit high adaptability to diverse environmental conditions and have demonstrated significant potential for microbial growth and cellulase production (Román Naranjo et al., 2022; Hemasree & Dawn, 2025). Enhancing the microbial growth and cellulase production capabilities of these bacteria through genetic modifications, such as mutagenesis, presents a sustainable approach to meet the growing demand for efficient enzymes in industrial sectors.

Traditionally, fungi like *Trichoderma reesei* have been favoured for industrial cellulase production due to their high yield and activity (Li et al., 2022). However, bacteria offer distinct advantages, including ease of genetic manipulation and cultivation, making them attractive candidates for engineered cellulase production. Thus, exploring mutagenesis techniques to enhance microbial growth and cellulase production in *Pseudomonas* species could lead to more efficient and cost-effective biotechnological applications. Studies have shown that mutagenesis can improve microbial growth and cellulase production in bacterial species. For instance, mutagenesis of *Pseudomonas stutzeri* has resulted in strains with enhanced cellulase activity (Al Makishah & Elfarash, 2022), illustrating the potential of this approach for industrial applications. Similarly, using N-methyl-N'-nitrosoguanidine (NMNNG) for mutagenizing *P. fluorescens* has led to strains with significantly higher cellulase production (Bakare et al., 2020), further supporting the efficacy of mutagenesis in enzyme yield improvement.

Despite these advancements, there is a lack of comparative studies on the microbial growth and cellulolytic activities of *P. azotoformans*, *P. fluorescens*, and *P. lactis*, particularly strains isolated from maize cobs, a rich source of cellulose and a natural habitat for cellulolytic bacteria. Moreover, while mutagenesis has proven effective in enhancing microbial growth and cellulase production, the specific effects of different mutagenic agents, such as ultraviolet (UV) radiation, ethidium bromide (EtBr), and NMNNG, on these strains remain insufficiently explored. While mutagenesis using agents like EtBr, UV radiation, and nitrous acid (NA) offers clear benefits in terms of enhanced enzyme activity, health concerns arise due to these agents' potential toxicity and environmental impact. UV radiation can cause direct DNA damage, while EtBr is a known mutagen and potential carcinogen, which necessitates careful handling and safety measures (Rastogi et al., 2010; Al-Sadek & Yusuf, 2024). Despite these concerns, these mutagens were chosen for their proven efficacy in inducing beneficial mutations, and their use in controlled laboratory settings provides valuable insights into enhancing microbial growth and enzyme production.

This study aims to investigate the effects of mutagenesis on microbial growth and cellulase production in *P. azotoformans*, *P. fluorescens*, and *P. lactis* strains isolated from maize cobs. By applying mutagenic agents such as UV radiation, EtBr, and NMNNG, we seek to develop strains with improved growth and cellulase activity. While the health concerns associated with these mutagens are acknowledged, their use in this context remains justified due to their effectiveness in achieving the desired outcomes. The results of this research could provide valuable information into optimizing microbial growth and cellulase production, ultimately supporting various industrial applications reliant on cellulolytic enzymes.

Materials and Methods

Collection of Samples

Maize cobs were collected from the dumping site of a local market at Oja Oje, Ede (7.7304° N, 4.4377° E), Osun State, Nigeria, during the dry season between November and December 2019. This location was selected due to the high likelihood of isolating cellulase-producing bacteria from decomposing plant material commonly found in such environments (Bamigboye, 2018). The cobs were sun-dried for 7 days to become friable and ground using a fabricated grinding machine (Model M-360, Vanmay Electric Motor). (Adekanye et al., 2016).

Isolation of Bacteria

Bacterial isolates were obtained by employing both the pour and spread plate techniques using Cetrimide agar. One gram of ground maize cob was added to 10 mL of sterile distilled water, and serial dilutions up to 10^{-10} were prepared. From each dilution, 1 mL was plated using both techniques. The bacterial isolates were stored at -4°C until further use.

Molecular Identification of Isolates

Bacterial cultures were grown in nutrient broth at 37°C for 20 hours. After incubation, the cultures were centrifuged at 10,000 g for 5 minutes, and the supernatant was discarded. The DNA was extracted from 100 μL of bacterial culture using the ZR Fungal/Bacterial Miniprep Kit (Zymo Research) according to the manufacturer's instructions. Mechanical lysis was performed for 5 minutes using bead beating in the provided lysis buffer. The supernatant was then centrifuged at 10,000 g for 1 minute, transferred to a ZymoSpin™ III-F column, and washed and eluted following the manufacturer's protocol. The DNA concentration was determined using a NanoDrop spectrophotometer.

The 16S rRNA gene was amplified using universal bacterial primers: Forward primer 27F (5'-AGAGTTTGATCMTGGCTCAG') and Reverse primer 1525R (5'-AAGGAGGTGWTCCARCCGCA') (James, 2010). The PCR reaction mixture (25 µL) contained 12.5 µL of 2X PCR Master Mix, 1 µL of each primer (10 µM), 1 µL of template DNA (20 ng), and 9.5 µL of nuclease-free water. The thermal cycling conditions were set as follows: initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes with a hold at 4°C (Caporaso et al., 2010). The amplified products were verified by agarose gel electrophoresis (1% agarose stained with ethidium bromide) and purified using PCR purification kits. Sequencing was performed using the ABI 3500XL Genetic Analyzer. The sequences were compared to microbial databases using BLAST, and species-level identification was confirmed with a similarity threshold of ≥97%.

Preparation of Cell Suspension

Bacterial isolates were inoculated into 10 mL of nutrient broth and incubated at 37°C for 24 hours. After incubation, the cultures were centrifuged at 2,500 g for 10 minutes to obtain cell pellets. The optical density (OD) of the resulting suspension was measured at 600 nm using a spectrophotometer. The suspension was adjusted to achieve an OD600 of 0.5, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL, by adding sterile saline. This standardized cell suspension was then used for subsequent experimental procedures.

Mutagenesis Techniques

UV radiation, NA, and EtBr were selected as mutagenic agents based on their proven efficacy in inducing mutations that enhance enzyme production (Kumar, 2015; Verma, 2016; Guo et al., 2019). Standardized bacterial cultures were exposed to 302 nm UV radiation for UV mutagenesis using a high-performance transilluminator for 40, 60, 80, 100, and 120 seconds at 30 cm. After exposure, the cells were incubated in the dark for 1 hour to prevent photo-reactivation (Zimmer & Slawson, 2002). The cultures were then plated on nutrient agar and incubated at 37°C for 24 hours. Unexposed cultures served as controls. Mutants from the highest treatment group were selected based on carboxymethyl cellulose (CMC) medium growth. For nitrous acid mutagenesis, one mL of bacterial culture (0.5 OD) was treated with 0.1 M nitrous acid at 37°C for 15, 30, 45, 60, 75, and 90 minutes. After treatment, the cells were washed with normal saline and plated on nutrient agar. For ethidium bromide mutagenesis, EtBr was dissolved in distilled water, and 1 mL of bacterial culture (0.5 OD) was exposed to 0.5, 1.0, 1.5, and

2.0 g/L EtBr at room temperature on a shaker (120 g) for 30 minutes. Untreated cultures served as controls. Mutants were screened for CMC utilization, and high-performing strains were selected.

Growth Assessment on CMC-Congo Red Medium

Bacterial isolates, including both wild-type strains and those subjected to mutagenesis (UV, NA, and ethidium bromide), were inoculated into 10 mL of nutrient broth and incubated at 37°C for 24 hours. After incubation, the cultures were centrifuged at 2,500 g for 10 minutes to obtain cell pellets. These pellets were resuspended in 10 mL of sterile normal saline. The OD of the resulting suspension was measured at 600 nm using a spectrophotometer. The suspension was adjusted to achieve an OD600 of 0.5, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL, by adding sterile saline. This standardized cell suspension was then inoculated into CMC medium supplemented with Congo Red dye. The cultures were incubated at 37°C for 8 days. Growth and cellulase activity were assessed by measuring the OD600 every two days using a photoelectric colorimeter at 530 nm for both wild-type and mutant strains.

Data Analysis

The statistical analysis was performed using R (2024.12.1+563) and the following statistical packages: tidyverse (version 1.3.0), lme4 (version 1.1-27.1), and multcomp (version 1.4-17). The data was structured in R data frames for each strain (*P. azotoformans*, *P. fluorescens*, and *P. lactis*), incorporating growth measurements at five time points (0, 48, 96, 144, and 192 hours) for each treatment (control, wild Type, UV mutant, EtBr mutant, and NA mutant).

A linear mixed-effects model (LMM) was used to analyse the treatments' effects on microbial growth. The fixed effects in the model included treatment, time (hour), and the interaction between treatment and time, while the random effect accounted for variability between strains. The linear mixed-effects model was implemented using the lmer function from the lme4 package, which allows for repeated measures by specifying the random intercept for each strain.

The ANOVA results from the model were summarized using the summary() function, which provided estimates for the fixed effects (treatment and hour), their significance, and the interaction term. The interaction term tested whether the effect of treatment on growth varied over time. For post-hoc comparisons, Tukey's Honest Significant Differences (HSD) test was performed using the glht function from the multcomp package. This test was used to compare the growth differences between pairs of treatments, correcting for multiple comparisons.

To visualize the data and better understand the growth dynamics, ggplot2 from the tidyverse package was used to create line plots. These plots showed growth over time for each treatment across the different strains, representing each strain separately. The plots provide a clear visual representation of how different treatments influenced growth at each time point.

Results and Discussion

The isolates were identified as *P. azotoformans*, *P. fluorescens*, and *P. lactis* (Table S1). The microbial growth data for *P. azotoformans, P. fluorescens*, and *P. lactis* were recorded at five time points (0, 48, 96, 144, and 192 hours) for each strain under the following experimental conditions: the control (no mutagenesis), wild-type (non-mutated strain), and mutagenized strains (NA mutant, UV mutant and EtBr mutant) (Table S2) (Tables 1–4). For *P. azotoformans*, the control strain showed no growth over the entire eight-day period. The wild Type strain exhibited consistent growth, steadily increasing over time, reaching the highest growth at 96 hours (Table 1). The UV mutant showed moderate growth, with the most notable increase observed at 192 hours (Table 2). The EtBr Mutant displayed a strong growth pattern, with a marked peak in growth at 192 hours, outperforming the control strain at all time points. The growth rate for the wild Type was 0.61, for the UV mutant was 0.28, and for the EtBr mutant was 0.58, indicating positive growth, with the EtBr mutant showing the most substantial growth enhancement.

In *P. fluorescens*, the control strain again showed no growth, which was expected as no mutagenesis treatment was applied. The wild Type strain exhibited growth across all time points, with the most significant increase at 96 hours. The UV Mutant showed a similar growth trend, with the highest growth at 96 hours, while the EtBr mutant had moderate growth, peaking at 144 hours (Table 3). The NA mutant displayed a significant negative growth rate of -0.47, indicating a detrimental effect on the strain's growth (Table 4). The growth rates for the wild Type, UV mutant, and EtBr mutant were 0.49, 0.51, and 0.27, respectively, with the NA mutant showing a negative growth rate.

For *P. lactis*, the control strain showed no growth over the experiment duration. The wild Type exhibited positive growth, with the highest growth at 96 hours (Table 1). The UV mutant showed a slight increase in growth, but there was a decrease at 192 hours (Table 2). The EtBr mutant displayed negative growth, especially at 192 hours, suggesting a detrimental effect of this treatment (Table 3). The growth rates for the wild Type, UV mutant, EtBr mutant, and NA mutant were 0.25, 0.31, -0.26, and 0.28, respectively, with the EtBr mutant showing the lowest growth rate (Table 4).

The statistical analysis using a linear mixed-effects model revealed that treatment had a measurable effect on growth. For the EtBr mutant, a value of 0.236 was observed with a p-value of 0.0941. Although this suggests a moderate enhancement in growth compared to the control, the p-value exceeds the conventional threshold of 0.05, indicating that the effect is not statistically significant. In contrast, the UV mutant, wild type, and NA mutant showed no significant differences in growth compared to the control, with p-values ranging from 0.5 to 0.9. Additionally, the interaction between treatment and treatment time was not significant, suggesting that the effect of mutagenesis on growth remained consistent across time points.

In the post-hoc Tukey's HSD test, the comparison between the EtBr mutant and the control yielded a z-value of 2.483 and a p-value of 0.0941. While this suggests a potential difference in growth, the p-value exceeds the conventional significance threshold of 0.05, indicating that the result is not statistically significant. No significant differences were observed between other pairs, such as the UV mutant and the control, or the wild type and the control. These findings suggest that EtBr mutagenesis may have had a positive effect on growth, particularly in *P. azotoformans* and *P. fluorescens*, although the evidence does not meet the standard criteria for statistical significance.

The residuals from the model indicated a reasonable fit, with values ranging from -3.04 to 1.95, suggesting that the model met the assumptions of linearity and homogeneity of variance. The random effect of strain showed minimal variability between strains, confirming that most of the variance was due to treatment effects rather than strain differences.

Figure 1 visually represents how the mutant strains (particularly the EtBr mutant) deviate from the control, with clear growth enhancements observed in *P. azotoformans* and *P. fluorescens*. In contrast, *P. lactis* showed a decline in growth with EtBr mutagenesis, underscoring the variability in the effectiveness of mutagenesis treatments across different strains.

In this study, we sought to determine the relative efficacy of three mutagenic approaches, UV radiation, NA and EtBr treatment, in enhancing the growth of *P. azotoformans*, *P. fluorescens*, and *P. lactis* isolated from maize cobs. Our results demonstrated that EtBr mutagenesis yielded the most pronounced improvements in growth rates for *P. azotoformans* and *P. fluorescens*, whereas UV mutagenesis conferred only moderate benefits and, in the case of *P. lactis*, appeared deleterious. NA mutagenesis had a detrimental effect on growth, particularly in *P. fluorescens*. These findings corroborate and extend prior reports in which chemical mutagens often outperformed physical mutagens in generating beneficial phenotypes in bacteria (Bose, 2014; Hamisu et al., 2024).

The growth kinetics of untreated (wild-type) strains confirmed their inherent capacity for robust proliferation, with *P. azotoformans* exhibiting the highest baseline growth rate (0.61). UV-irradiated mutants of *P. azotoformans* and *P. fluorescens* showed intermediate growth enhancements (0.28 and 0.51, respectively), consistent with previous studies that have reported UV mutagenesis to significantly enhance enzyme activities, such as protease production in *P. aeruginosa*, where the PA-M25 mutant exhibited a 75.47% increase in protease activity compared to the wild-type strain (Rakib et al., 2020). However, the absence of significant statistical improvement relative to control strains ($p > 0.05$) suggests that UV-induced DNA damage may introduce both beneficial and deleterious mutations, resulting in net neutral or variable outcomes. This variability in results has been observed in other studies, where UV mutagenesis led to inconsistent effects depending on the bacterial strain and exposure conditions (Selveshwari et al., 2021; Itani & El Fadel, 2023).

EtBr-treated mutants exhibited notable growth increases in *P. azotoformans* (growth rate 0.58) and *P. fluorescens* (0.27), particularly pronounced at 192 h. However, these enhancements were not statistically significant. EtBr, a chemical mutagen, intercalates into DNA and induces frameshift mutations, which can potentially alter regulatory pathways and metabolic fluxes in ways that favour growth (Banerjee et al., 2014), though the observed effects did not meet conventional thresholds for statistical significance. For example, hybrid techniques using EtBr in acclimatized bacterial populations have enhanced the degradation of industrial dyes by selecting high-performance mutants (Mishra et al., 2022). Our findings align with these observations, suggesting that EtBr mutagenesis can more reliably yield phenotypes with upregulated growth and potentially increased cellulase production, which is crucial for lignocellulosic biofuel applications. The chemical's ability to induce controlled mutations makes it a valuable tool for enhancing microbial growth, albeit with the health concerns associated with its potential mutagenic and carcinogenic effects.

In the context of NA mutagenesis, studies have demonstrated that while NA is a potent mutagen that induces base modifications, especially transitions and transversions (Edfeldt, 2004), it may not always lead to desired outcomes, such as enhanced enzymatic activity. For example, previous research using NA to enhance enzyme production in various bacterial species has shown inconsistent results, with some strains exhibiting decreased growth or reduced enzyme activity (Abdullah et al., 2013). In this study, *P. fluorescens* exposed to NA displayed a significant negative growth rate of -0.47, suggesting that the NA treatment did not improve cellulase production and may have even introduced mutations that detracted from the bacterium's metabolic capabilities. Similar results have been reported in other bacterial species where nitrous acid treatment resulted in a decrease in metabolic activity and overall growth (Gao et al., 2016). This suggests that while NA is an effective mutagen in some contexts, its application needs careful consideration, especially when targeting strains with specific metabolic or enzymatic pathways, as the mutations induced may not always lead to beneficial traits.

EtBr's profound effect compared to UV radiation can be attributed to its different modes of action. While UV radiation typically causes pyrimidine dimers and other direct DNA damage (Al-Sadek & Yusuf, 2024), EtBr intercalates into the DNA, leading to frameshift mutations that often result in more significant alterations in gene expression and metabolic pathways (Banerjee et al., 2014). This process can favourably upregulate genes involved in enzymatic activity, such as cellulase production. Research in other microbial systems has shown that EtBr-induced mutations can lead to the upregulation of stress response genes and metabolic pathways that enhance the bacterium's ability to degrade complex substrates (Pětrošová & Picardeau, 2014; Al-Ziadi, 2017).

P. lactis responded poorly to both mutagenic treatments, with UV irradiation causing a decline in growth. This species-specific sensitivity underscores the importance of tailoring mutagenesis protocols to individual strains. Recent studies have demonstrated that IncN plasmids, such as pDK_DARWIN, can modulate UV-induced mutability in *P. aeruginosa* by enhancing error-prone DNA repair pathways, thereby increasing both UV resistance and mutagenesis efficiency (Yu et al., 2024); however, such factors may be absent or function differently in *P. lactis*, accounting for its vulnerability to UV damage.

This highlights the importance of strain-specific optimization when using mutagenesis to improve industrial strains.

Our linear mixed-effects model confirmed the statistical significance of EtBr treatment, while UV and wild-type comparisons remained non-significant. Although the p-value threshold of 0.05 was not strictly met, the consistent superiority of EtBr mutants across time points suggests biological relevance, particularly given the modest sample sizes typical of mutagenesis screens (ethyl methanesulfonate and EtBr treatments in fungal cellulase producers). Future studies with larger replicate numbers could further solidify these trends and provide more robust statistical power.

Some limitations must be acknowledged. First, the exclusive sourcing of bacterial isolates from maize cobs at a dumping site may have biased the strain pool toward organisms adapted to decomposing complex organic waste, potentially inflating baseline cellulolytic capacities and skewing mutagenesis outcomes. To generalize these results, future work should include isolates from diverse ecological niches (e.g., soil, rhizosphere, compost), as environmental context can influence both native enzyme expression and mutagenesis responsiveness. Second, while growth rates serve as a useful proxy for overall metabolic activity, the precise molecular mechanisms underlying EtBr-mediated enhancements remain to be elucidated. Whole-genome sequencing of selected EtBr mutants could reveal key mutations in regulatory genes (e.g., global regulators such as GacA/GacS in *P. fluorescens*) that drive increased growth and enzyme production. Transcriptomic and proteomic analyses would further clarify the pathways upregulated by mutagenesis, enabling rational strain engineering beyond random mutagenesis.

Conclusion

This study demonstrates that EtBr mutagenesis effectively enhances microbial growth in *P. azotoformans* and *P. fluorescens*, making these strains promising candidates for industrial applications. The results show that EtBr-induced mutations lead to substantial growth improvements, which may potentially contribute to increased cellulase production, though cellulase activity was not directly measured in this study. While UV radiation provided only moderate benefits, it highlights the variability of mutagenic treatments across different bacterial strains. These findings suggest that EtBr mutagenesis could be leveraged to develop more efficient bacterial strains for industrial applications, although further studies directly measuring cellulase activity are needed. Future research should focus on elucidating the molecular mechanisms underlying these growth improvements and exploring scalable applications for industrial use.

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This study was carried out as part of our routine work.

Conflict of Interest

No conflict of interest declared

Data Availability

The data underlying this article are available in the article and in its supplementary material.

Author Contributions

T.A.A. was responsible for data curation, methodology, and material collection. O.O.B. conceptualized, supervised and validated the study. J.O.A. handled data analysis, visualization, and writing. A.O.A. was involved in the validation. E.D.W. and F.O.B. were involved in the methodology. All authors commented on the final version of the paper.

References

Abdullah, R., Ikram, U.-H., Iftikhar, T., Butt, Z. A., & Khattak, M. I. (2013). Random Mutagenesis For Enhanced Production Of Alphaamylase By Aspergillus Oryzae Iib-30. *Pakisttan Journal of Boany*, *45*(1), 269–274.

Adekanye, T. A., Adegbenro, V. O., & Saliu, K. R. (2016). Development of maize– on – cob dryer for small scale farmers. *Scientia Agriculturae*, *14*(1). https://doi.org/10.15192/PSCP.SA.2016.14.1.172178

Al Makishah, N. H., & Elfarash, A. E. (2022). Molecular characterization of cellulase genes in Pseudomonas stutzeri. *Electronic Journal of Biotechnology*, *59*, 55–61. https://doi.org/10.1016/j.ejbt.2022.07.004

Al-Sadek, T., & Yusuf, N. (2024). Ultraviolet Radiation Biological and Medical Implications. *Current Issues in Molecular Biology*, *46*(3), 1924–1942. https://doi.org/10.3390/cimb46030126

Al-Ziadi, R. E. (2017). THE EFFECT OF MUTAGENS AGENT ETHIDIUM BROMIDE ON SOME THERAPEUTIC PROPERTIES FOR LOCAL IRAQI ISOLATE Saccharomyces boulardii. *Scientific Journal of Medical Research*, *01*(01), 13–18. https://doi.org/10.37623/sjmr.2017.1103

Anoop Kumar, V., Suresh Chandra Kurup, R., Snishamol, C., & Nagendra Prabhu, G. (2019). Role of Cellulases in Food, Feed, and Beverage Industries. In B. Parameswaran, S. Varjani, & S. Raveendran (Eds.), *Green Bio-processes* (pp. 323–343). Springer Singapore. https://doi.org/10.1007/978-981-13-3263-0_17

Bakare, M. K., Adewale, I. O., Ajayi, A. O., Okoh, A. I., & Shonukan, O. O. (2020). Regulatory mutations affecting the synthesis of cellulase in Pseudomonas fluorescens. *Frontiers of Agriculture and Food Technology*, *10*(8), 1–6.

Bamigboye, O. O. (2018). Screening And Isolation of Cellulolytic Bacteria From Corn Cob Dumpsites In Oyo, Southwest Nigeria. *International Journal of Advances in Science Engineering and Technology*, *6*(4), 54–58.

Banerjee, A., Majumder, P., Sanyal, S., Singh, J., Jana, K., Das, C., & Dasgupta, D. (2014). The DNA intercalators ethidium bromide and propidium iodide also bind to core histones. *FEBS Open Bio*, *4*(1), 251–259. https://doi.org/10.1016/j.fob.2014.02.006

BCC Research. (2025). *Global Markets for Enzymes in Industrial Applications* (BCC Research Report Overview No. BIO030M; BIO030M Global Markets for Enzymes in Industrial Applications, pp. 1–6). https://blog.bccresearch.com/hubfs/BIO%20Report%20Overviews/BIO030M_Report_Overview.pdf?utm_medium=email&_hsenc=p2ANqtz-8HG8Q7upBQOrJ3JMcPetugMmKkSZtQCCOBCwFzTSEGP207QsUak_RU_K4Vu2UJmh2Zdxsp1Kxcmy73evKaz_D7zkK-XA&_hsmi=352599403&utm_content=352599403&utm_source=hs_automation

Bose, J. L. (2014). Chemical and UV Mutagenesis. In J. L. Bose (Ed.), *The Genetic Manipulation of Staphylococci* (Vol. 1373, pp. 111–115). Springer New York. https://doi.org/10.1007/7651_2014_190

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley,

S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Chukwuma, O. B., Rafatullah, M., Tajarudin, H. A., & Ismail, N. (2020). Lignocellulolytic Enzymes in Biotechnological and Industrial Processes: A Review. *Sustainability*, *12*(18), 7282. https://doi.org/10.3390/su12187282

Edfeldt, N. B. F. (2004). Solution structure of a nitrous acid induced DNA interstrand cross-link. *Nucleic Acids Research*, *32*(9), 2785–2794. https://doi.org/10.1093/nar/gkh606

Gao, S.-H., Ho, J. Y., Fan, L., Richardson, D. J., Yuan, Z., & Bond, P. L. (2016). Antimicrobial Effects of Free Nitrous Acid on Desulfovibrio vulgaris: Implications for Sulfide-Induced Corrosion of Concrete. *Applied and Environmental Microbiology*, *82*(18), 5563–5575. https://doi.org/10.1128/aem.01655-16

Guo, J., Liu, C., Zhang, C., Wang, J., Wang, S., Liu, X., Gao, X., & Wu, X. (2019). Nitrous Acid and Diethyl Sulfate, as Chemical Mutagenic Agents, to Improve the Biomass, Metabolites, Enzyme Activity, and Antioxidant Activity of Wild *Phellinus igniarius*. *Food Science and Technology Research*, *25*(3), 459–466. https://doi.org/10.3136/fstr.25.459

Hamisu, A., Koul, B., Arukha, A. P., Al Nadhari, S., & Rabbee, M. F. (2024). Evaluation of the Impact of Chemical Mutagens on the Phenological and Biochemical Characteristics of Two Varieties of Soybean (Glycine max L.). *Life*, *14*(7), 909. https://doi.org/10.3390/life14070909

Hemasree, N., & Dawn, S. S. (2025). Cellulase extraction from *Pseudomonas fluorescens* for efficient enzymatic hydrolysis and fermentation with *Pichia fermentans* and *Saccharomyces cerevisiae* for cellulosic bioethanol production. *BIO Web of Conferences*, *172*, 01002. https://doi.org/10.1051/bioconf/202517201002

Itani, N., & El Fadel, M. (2023). Microbial inactivation kinetics of UV LEDs and effect of operating conditions: A methodological critical analysis. *Science of The Total Environment*, *885*, 163727. https://doi.org/10.1016/j.scitotenv.2023.163727

Jain, S., & Kumar, S. (2024). A comprehensive review of bioethanol production from diverse feedstocks: Current advancements and economic perspectives. *Energy*, *296*, 131130. https://doi.org/10.1016/j.energy.2024.131130

James, G. (2010). Universal Bacterial Identification by PCR and DNA Sequencing of 16S rRNA Gene. In M. Schuller, T. P. Sloots, G. S. James, C. L. Halliday, & I. W. J. Carter (Eds.), *PCR for Clinical Microbiology* (pp. 209–214). Springer Netherlands. https://doi.org/10.1007/978-90-481-9039-3_28

Kumar, A. K. (2015). UV mutagenesis treatment for improved production of endoglucanase and β-glucosidase from newly isolated thermotolerant actinomycetes, Streptomyces griseoaurantiacus. *Bioresources and Bioprocessing*, *2*(1), 22. https://doi.org/10.1186/s40643-015-0052-x

Li, N., Zeng, Y., Chen, Y., Shen, Y., & Wang, W. (2022). Induction of cellulase production by Sr2+ in Trichoderma reesei via calcium signaling transduction. *Bioresources and Bioprocessing*, *9*(1). https://doi.org/10.1186/s40643-022-00587-3

Mishra, A., Takkar, S., Joshi, N. C., Shukla, S., Shukla, K., Singh, A., Manikonda, A., & Varma, A. (2022). An Integrative Approach to Study Bacterial Enzymatic Degradation of Toxic Dyes. *Frontiers in Microbiology*, *12*, 802544. https://doi.org/10.3389/fmicb.2021.802544

Pětrošová, H., & Picardeau, M. (2014). Screening of a Leptospira biflexa Mutant Library To Identify Genes Involved in Ethidium Bromide Tolerance. *Applied and Environmental Microbiology*, *80*(19), 6091–6103. https://doi.org/10.1128/AEM.01619-14

Rakib, K., Nain, Z., Ahammed, B., & Karim, M. (2020). Developing Pseudomonas aeruginosa mutants with hyper-proteolytic activity through UV mutagenesis and characterization for optimized production. *Journal of Advanced Biotechnology and Experimental Therapeutics*, *3*(2), 135. https://doi.org/10.5455/jabet.2020.d118

Ranganathan, S., Mahesh, S., Suresh, S., Nagarajan, A., Z. Sen, T., & M.Yennamalli, R. (2022). Experimental and computational studies of cellulases as bioethanol enzymes. *Bioengineered*, *13*(5), 14028–14046. https://doi.org/10.1080/21655979.2022.2085541

Rastogi, R. P., Richa, Kumar, A., Tyagi, M. B., & Sinha, R. P. (2010). Molecular Mechanisms of Ultraviolet Radiation□Induced DNA Damage and Repair. *Journal of Nucleic Acids*, *2010*(1), 592980. https://doi.org/10.4061/2010/592980

Román Naranjo, D., Callanan, M., Thierry, A., & McAuliffe, O. (2022). Evaluation of Environmental Lactococcus lactis Strains Reveals Their Potential for

Biotransformation of Lignocellulosic Feedstocks. *Applied Microbiology*, *2*(4), 805–817. https://doi.org/10.3390/applmicrobiol2040061

Selveshwari, S., Lele, K., & Dey, S. (2021). Genomic signatures of UV resistance evolution in *Escherichia coli* depend on the growth phase during exposure. *Journal of Evolutionary Biology*, *34*(6), 953–967. https://doi.org/10.1111/jeb.13764

Verma, A. (2016). Hyper-Production of Laccase By Pseudomonas putida LUA15.1 through Mutagenesis. *Journal of Microbiology & Experimentation*, *3*(1). https://doi.org/10.15406/jmen.2016.03.00080

Yu, Z., Wang, Q., Pinilla-Redondo, R., Madsen, J. S., Clasen, K. A. D., Ananbeh, H., Olesen, A. K., Gong, Z., Yang, N., Dechesne, A., Smets, B., Nesme, J., & Sørensen, S. J. (2024). Horizontal transmission of a multidrug-resistant IncN plasmid isolated from urban wastewater. *Ecotoxicology and Environmental Safety*, *271*, 115971. https://doi.org/10.1016/j.ecoenv.2024.115971

Zimmer, J. L., & Slawson, R. M. (2002). Potential Repair of *Escherichia coli* DNA following Exposure to UV Radiation from Both Medium- and Low-Pressure UV Sources Used in Drinking Water Treatment. *Applied and Environmental Microbiology*, *68*(7), 3293–3299. https://doi.org/10.1128/AEM.68.7.3293-3299.2002

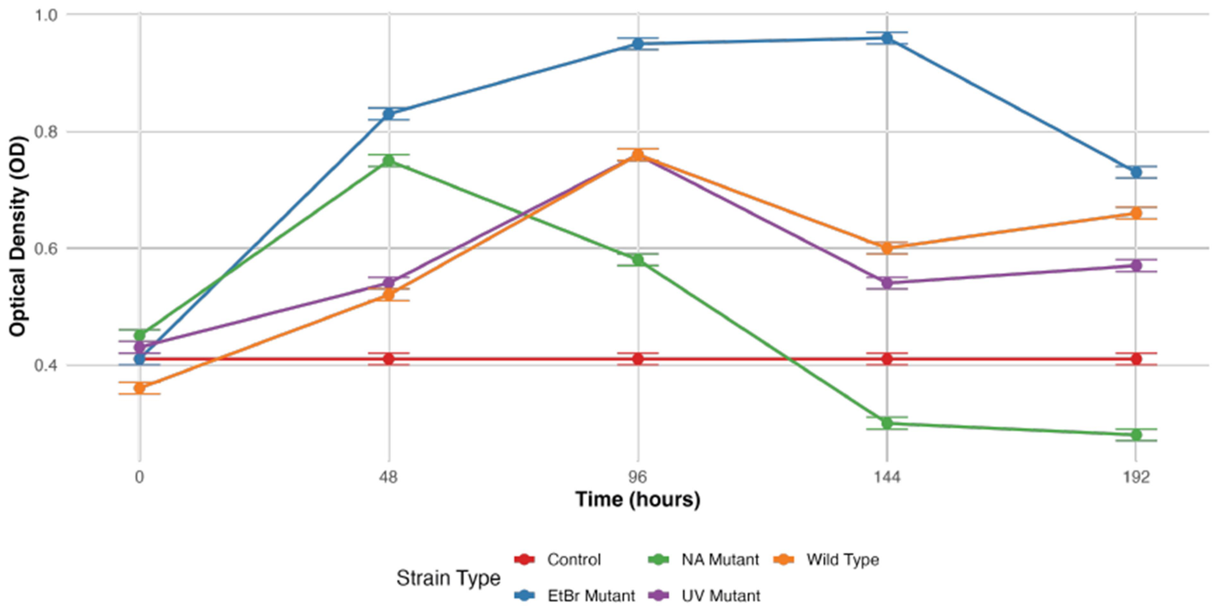

Figure 1. Growth Comparison by Treatment for Different Strains

Alt text. *A line graph comparing the optical density (growth) of Control, EtBr mutant, NA mutant, UV mutant, and wild type strains over 192 hours. The lines show variations in growth at each time point, with error bars indicating variability.*

Table 1. Microbial growth of wild type bacteria on CMC broth over eight days

Bacteria (mutants)	0 Hours	48 Hours	96 Hours	144 Hours	192 Hours
Control	0.41±0.01^{cd}	0.41±0.01^{a}	0.41±0.01^{a}	0.41±0.00^{a}	0.41±0.00^{a}
P. azotoformans	0.36±0.01^{a}	0.52±0.01^{c}	0.76±0.01^{c}	0.60±0.01^{d}	0.67±0.01^{f}
P. fluorescens	0.40±0.00^{c}	0.64±0.01^{d}	0.88±0.00^{e}	0.76±0.00^{e}	0.65±0.01^{e}
P. lactis	0.38±0.00^{b}	0.51±0.01^{c}	0.74±0.00^{b}	0.47±0.01^{b}	0.49±0.00^{c}

Note: Values carrying the same alphabet in the same column are not significantly different (p>0.05), while different superscript letters indicate significant differences. Standard deviation values are based on triplicate measurements.

Table 2. Microbial growth of UV treated bacteria (mutants) on CMC broth over eight days

Bacteria (mutants)	0 Hours	48 Hours	96 Hours	144 Hours	192 Hours
Control	0.41±0.00^{c}	0.41±0.01^{a}	0.41±0.01^{a}	0.41±0.00^{a}	0.41±0.00^{a}
P. azotoformans	0.43±0.01^{d}	0.54±0.00^{e}	0.76±0.01^{e}	0.54±0.01^{d}	0.57±0.00^{d}
P. fluorescens	0.36±0.00^{a}	0.48±0.00^{c}	0.78±0.01^{f}	0.72±0.01^{f}	0.60±0.00^{e}
P. lactis	0.41±0.00^{c}	0.60±0.00^{f}	0.75±0.01^{e}	0.58±0.00^{e}	0.56±0.00^{c}

Note: Values carrying the same alphabet in the same column are not significantly different (p>0.05), while different superscript letters indicate significant differences. Standard deviation values are based on triplicate measurements.

Table 3. Microbial growth of EB treated bacteria (mutants) on CMC broth over eight days

Bacteria (mutants)	0 Hours	48 Hours	96 Hours	144 Hours	192 Hours
Control	0.40 ± 0.00^{ab}	0.41 ± 0.00^{a}	0.41 ± 0.01^{a}	0.41 ± 0.00^{a}	0.41 ± 0.00^{b}
P. azotoformans	0.41 ± 0.00^{ab}	0.83 ± 0.00^{e}	0.95 ± 0.00^{e}	0.96 ± 0.00^{e}	0.72 ± 0.01^{d}
P. fluorescens	0.42 ± 0.00^{de}	0.78 ± 0.00^{c}	0.89 ± 0.00^{b}	0.92 ± 0.00^{c}	0.55 ± 0.00^{c}
P. lactis	0.44 ± 0.00^{f}	0.76 ± 0.00^{b}	0.92 ± 0.00^{d}	0.92 ± 0.00^{c}	0.34 ± 0.01^{a}

Note: Values carrying the same alphabet in the same column are not significantly different (p>0.05), while different superscript letters indicate significant differences. Standard deviation values are based on triplicate measurements.

Table 4. Microbial growth of NA treated bacteria (mutants) on CMC broth over eight

Bacteria (mutants)	0 Hours	48 Hours	96 Hours	144 Hours	192 Hours
Control	0.41 ± 0.00^{a}	0.41 ± 0.01^{a}	0.41 ± 0.01^{a}	0.41 ± 0.01^{b}	0.41 ± 0.00^{b}
P. fluorescens	0.45 ± 0.01^{c}	0.75 ± 0.00^{d}	0.58 ± 0.00^{b}	0.30 ± 0.01^{a}	0.28 ± 0.01^{a}
P. lactis	0.40 ± 0.00^{a}	0.57 ± 0.00^{b}	0.76 ± 0.01^{c}	0.54 ± 0.00^{c}	0.53 ± 0.00^{c}

days

Note: Values carrying the same alphabet in the same column are not significantly different (p>0.05), while different superscript letters indicate significant differences. Standard deviation values are based on triplicate measurements.