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Research Article

CHARACTERIZATION AND DEGRADATION OF PHARMACEUTICAL PRODUCT LEVOFLOXACIN BY SELECTED BACTERIAL ISOLATES

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Abstract

Aim: Effective bioremediation techniques are required to lessen the ecological impact of the commonly used fluoroquinolone antibiotic levofloxacin due to its environmental persistence.

Method: Nine of the 11 bacterial isolates that were obtained from soil samples using an enrichment technique in this investigation showed resistance to levofloxacin concentrations as high as 500 ppm. Isolates LFX-2a, LFX-8a, and LFX-10a shown robust growth at all tested concentrations, according to screening for Maximum Tolerance Level (MTL).

Results: After 72 hours, LFX-10a had the highest degradation rate, at 71.24%. At 50.24%, the consortium DEF (LFX-2a, LFX-8a, and LFX-10a) likewise displayed notable degradation. Seven of the isolates were motile and varied in their endospore and capsule formation, according to morphological characterization, which showed a mixture of rod-shaped bacteria that were both Gram-positive and Gram-negative. Numerous metabolic capacities, such as universal catalase synthesis, nitrate reduction in seven isolates, and varied responses in IMViC and sugar fermentation tests, were validated by biochemical analyses.

Conclusion: Although extended exposure may cause stress, growth tests revealed that the peak bacterial activity occurred between 120 and 144 hours, indicating levofloxacin metabolism. These results demonstrate the levofloxacin bioremediation capability of LFX-10a and the DEF consortia, laying the groundwork for additional research into their enzymatic processes and useful applications in environmental management.

Keywords: Levofloxacin, Biological degradation, Bacterial isolates, Antibiotic resistance

1. Introduction

The rising prevalence of pharmaceutical contaminants in the environment, especially antibiotics, has generated considerable apprehension due to their capacity to damage ecosystems and foster antibiotic resistance. Levofloxacin, a third-generation fluoroquinolone antibiotic, is extensively utilised for the treatment of bacterial infections in both humans and animals (1). Nonetheless, its inadequate metabolism and incorrect disposal lead to its persistence in wastewater, surface water, and soil . so contributing to environmental contamination. The obstinate properties of levofloxacin, due to its intricate chemical composition, make standard wastewater treatment techniques inadequate, requiring alternative strategies such microbial breakdown. The biological degradation of pharmaceutical contaminants by specific bacterial isolates is a promising, environmentally sustainable approach by utilizing the metabolic functions of microorganisms to decompose complex chemicals (2). Recent research has revealed bacteria from soil and wastewater that may degrade fluoroquinolones via enzymatic pathways, underscoring their potential for bioremediation.

The extensive utilisation of antibiotics, including levofloxacin, has resulted in their continual accumulation in environmental matrices, such as wastewater, surface water, and soil, hence presenting considerable ecological and public health hazards (3).

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Levofloxacin, a third-generation fluoroquinolone antibiotic, is widely utilised for the treatment of bacterial infections owing to its broad-spectrum efficacy. Nonetheless, its incomplete metabolism in humans and animals, along with insufficient disposal methods, leads to its environmental discharge, hence fostering antibiotic resistance and disturbing microbial ecosystems. The chemical stability of levofloxacin restricts the effectiveness of traditional therapeutic approaches, necessitating the investigation of biological degradation as a viable alternative. Isolated bacteria, especially from polluted soils and wastewater, exhibit the capacity to breakdown intricate medicinal molecules such as levofloxacin via specialised metabolic pathways (4). Comprehending the physical and biochemical traits of these bacteria is essential for selecting strains with superior degradative ability and clarifying the mechanisms that underpin their activity. This study examines the morphological and biochemical characteristics of bacterial isolates that may degrade levofloxacin, as well as their degradation efficiency, to evaluate their potential for bioremediation. This research seeks to elucidate microbial processes by combining microbial characterization with degradation investigations, thereby aiding in the formulation of efficient, environmentally sustainable ways to mitigate levofloxacin pollution.

Methodology

Sample collection

A total of 10 different soil samples have been collected from different localities in Gwalior which is situated in 26.22° North Latitude and 78.18° East Longitude in Madhya Pradesh. Temperature and pH of soil samples were taken at the time of sampling with the help of thermometer and pH meter respectively. All samples were kept at 4°C till further processing.

Enrichment and acclimatization of bacterial isolates

The bacterial culture obtained were enriched using a sequence of dilution and incubation processes. One gram (1 g) of collected soil specimen was suspended in 9 mL of sterile distilled water and stirred for one minute. The homogenised soil extract solution was diluted by combining 1 mL of the solution with 9 mL of sterile filtered water. Subsequently, 1 mL of the diluted solution was incorporated into 49 mL of sterile Minimal Salt Medium, which contained a concentration of 20 µg/mL of each antibiotic, Levofloxacin (LFX), serving as the only carbon source. The mixture was incubated at 30°C, agitated at 150 rpm for 7 days until an optimal optical density (OD) was attained. The objective of acclimatization was to acquire bacterial strains with elevated tolerance and degradation capacity for specific antibiotics (6).

Isolation and purification of enriched bacterial isolates

Isolation and purification were conducted following enrichment and acclimatization. The solution from the last acclimatization flask was serially diluted with sterile distilled water three times (i.e., 1:1000 dilution), distributed onto LB agar medium, and incubated at 30°C for 48 hours. The streak plate method was employed to separate and purify microorganisms exhibiting distinct colony morphologies originally. The unadulterated bacterial strains were preserved at -20°C in a nutrient broth with 15% glycerol (8).

Screening of bacterial isolates for Maximum Tolerance Level (MTL) of selected antibiotic Levofloxacin (LFX)

Malik and Jaiswal in 2000 (5) investigated the ability of bacterial strains to proliferate at increasing antibiotic doses using a modified technique. To assess Maximum Tolerance, bacterial isolates were aseptically streaked on LB Agar plates supplemented with escalating antibiotic concentrations (50, 100, 200, 300, 400, and 500 ppm of each selected antibiotic) and monitored for growth after 24-48 hours of incubation at 37°C. The Maximum Tolerance Level (MTL) to each antibiotic that promotes bacterial growth was determined for each bacterial isolate.

Growth study of bacterial isolates at varying concentration of selected antibiotics

This study was conducted to determine the maximum growth of antibiotic Levofloxacin (LFX)-degrading bacteria in MSM containing variable antibiotic concentrations at different time intervals. Antibiotic concentrations were measured at 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm. The selected bacterial isolates' growth rate was regularly checked indirectly by turbidity measuring assay (OD at 600nm) at 24-hour intervals using a UV visible spectrophotometer.

Morphological and Biochemical characterization and identification of selected antibiotics degrading bacterial isolates

For this, Gram staining, endospore staining, capsule staining and motility tests were performed for Levofloxacin (LFX) degrading bacterial isolates.
To study the biochemical properties of selected pharmaceutical products Levofloxacin (LFX), degrading bacterial isolates the following tests catalase, oxidase, casein hydrolysis, Indole synthesis, methyl red, citrate utilization, and nitrate reduction tests were performed according to the standard protocols (7).

Quantitative degradation of Levofloxacin (LFX) by selected bacterial isolates

For the purpose of studying the breakdown of particular antibiotics, the chosen bacterial culture was cultivated in nutritional broth at 37°C for 24 hours while being continuously shaken at 150 rpm. To enable the bacteria to reach the log (exponential) growth phase, incubate the culture for 18 to 24 hours at 37°C while shaking it at 150 to 200 rpm.

For seven days, the inoculum was incubated at 37°C with shaking (150 rpm) in 50 mL of sterile nutritional broth that contained 50 mg/L of specific pharmaceutical goods, such as Levofloxacin (LFX). Five millilitres of samples were taken out of the culture media for examination at regular intervals of 0 hr and 72 hours. To get rid of any detritus and bacterial organisms, the samples were centrifuged for five minutes at 10,000 rpm. To determine how much antibiotic was still present in the solution, the supernatants were examined using UV-visible spectrophotometry(9).

A UV-Visible spectrophotometer was used to measure the absorbance of the supernatant at a different range for each antibiotic Levofloxacin (290). By comparing the absorbance readings to a standard calibration curve derived from known antibiotic concentrations (0-100 mg/L), the antibiotic concentration was ascertained.

Determination of % Degradation: The degradation percentage of antibiotic was calculated based on the initial and final concentrations of the drug:

$$\%\ Degradation = \frac{(C_0 - C_t)}{C_0} \times 100$$

where: C_0- is the initial concentration of antibiotic (at day 0), Ct -is the concentration of antibiotic at time

Statistical analysis

One way ANOVA was used to analyse the data using SPSS 16.0 software. Microsoft Excel 2016 was used to compute the mean and standard deviation for the tests, which were carried out in three repetitions.

Result and discussion

Sample collection

Sample collection was done from non-contaminated sites like normal dumping sites, suspected sites nearby region of pharmaceutical industries sites. Temperature of different samples varied in the range from 19°C to 40°C. The pH of the samples also varied from 6.50-8.30.

Enrichment and isolation of antibiotic degrading bacterial isolates

The enrichment process was used to isolate and select bacteria that could have antibiotic degradation potential. An increase in turbidity and a change in the colour of the medium were the indicators of bacterial growth and antibiotic utilization when compared to uninoculated control. Hence, a total of 11 bacterial isolates were obtained from the soil samples by the enrichment culture method after a series of sub-cultures of four day intervals incubated at 37ºC and 120 rpm(10).

Screening of bacterial isolates for Maximum Tolerance Level (MTL) of Levofloxacin (LFX)

Table 1 presents the Maximum Tolerance Level (MTL) of levofloxacin (LFX) degrading bacterial isolates at six successive concentrations of the antibiotic, ranging from 50 ppm to 500 ppm. The data indicate variability in the tolerance levels among the bacterial isolates. Notably, LFX-2a, LFX-5a, LFX-8a, LFX-8b and LFX-10a exhibited the highest tolerance, demonstrating sustained growth at all concentrations, with some reduction in intensity at higher levels (500ppm).

These isolates maintained at least moderate growth (++), even at 500 ppm, suggesting a strong adaptive capability or inherent resistance to levofloxacin. Conversely, isolates such as LFX-6a and LFX-4a exhibited lower tolerance, with LFX-6a ceasing growth at 400 ppm and LFX-4a at 500 ppm. Among the isolates showing moderate tolerance, LFX-1a, LFX-3a, LFX-7a, and LFX-9a displayed growth reduction beyond 300 ppm, with only mild growth observed at 400 and 500 ppm. This indicates limited but present ability to survive under increasing levofloxacin concentrations. Result concluded that out of 11 levofloxacin degrading bacterial isolates only 09 isolates were able to tolerate upto 500ppm concentration of levofloxacin, hence only 09 isolates (LFX-1a, LFX-2a, LFX-3a, LFX-5a, LFX-7a, LFX-8a, LFX-8b, LFX-9a, LFX-10a**)** were used for further study(11).

Table 1: Maximum Tolerance Level (MTL) of Levofloxacin (LFX) degrading bacterial isolates at six successive concentrations of antibiotic

Bacterial isolates	Bacterial growth at varying concentration of Levofloxacin (LFX)					
	50 ppm	100 ppm	200ppm	300ppm	400ppm	500ppm
LFX-1a	++	++	++	++	+	+
LFX-2a	+++	+++	+++	++	++	++
LFX-3a	+++	++	++	++	+	+
LFX-4a	++	++	++	+	+	-
LFX-5a	+++	+++	+++	++	++	+
LFX-6a	++	++	+	+	-	-
LFX-7a	+++	++	++	++	+	+
LFX-8a	++	++	++	++	++	++
LFX-8b	+++	++	++	++	++	++
LFX-9a	++	++	++	++	+	+
LFX-10a	+++	+++	+++	++	++	+

(+)- Mild growth, (++)- Moderate growth,(+++)- Maximum growth and (-)- No growth

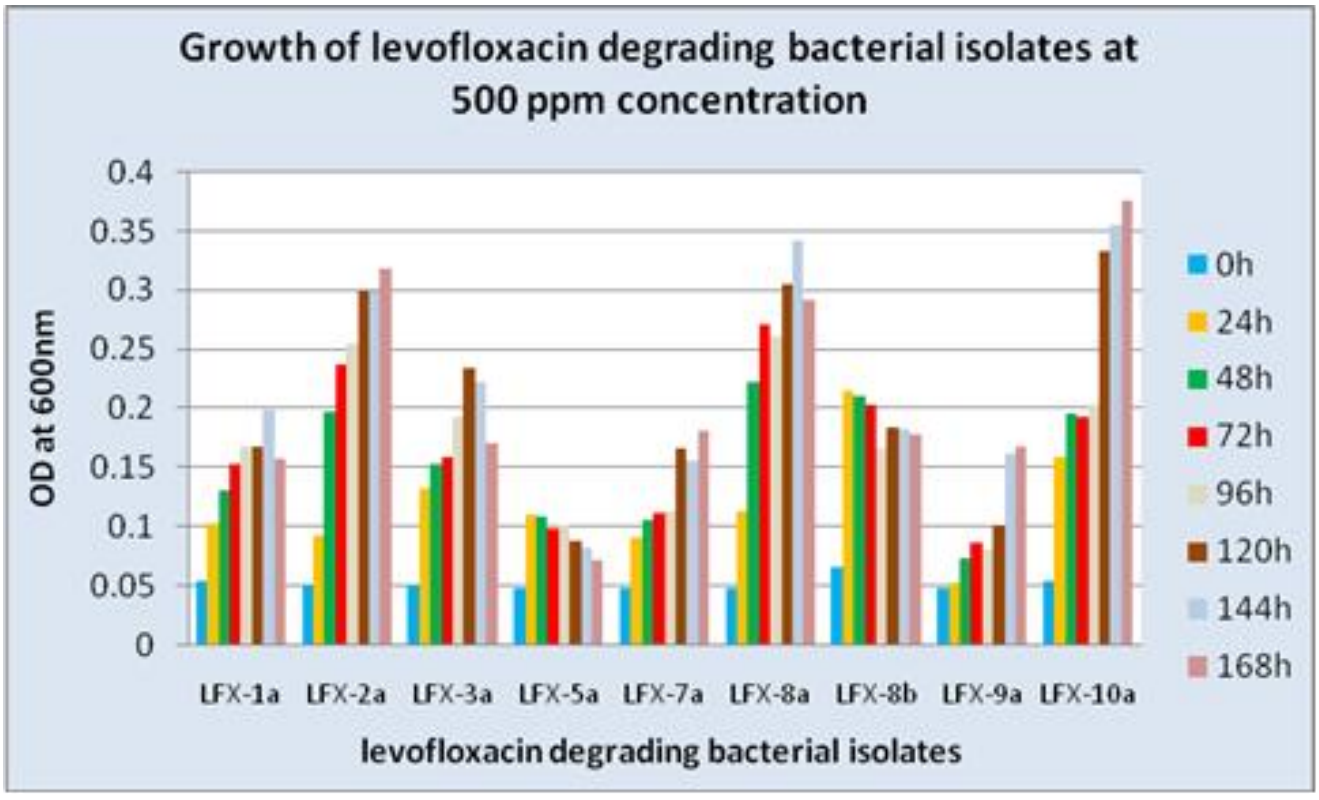

Figure 1: Relationship between growth and duration of incubation of levofloxacin degrading bacterial isolate at 500 ppm concentration of levofloxacin (OD at 600nm)

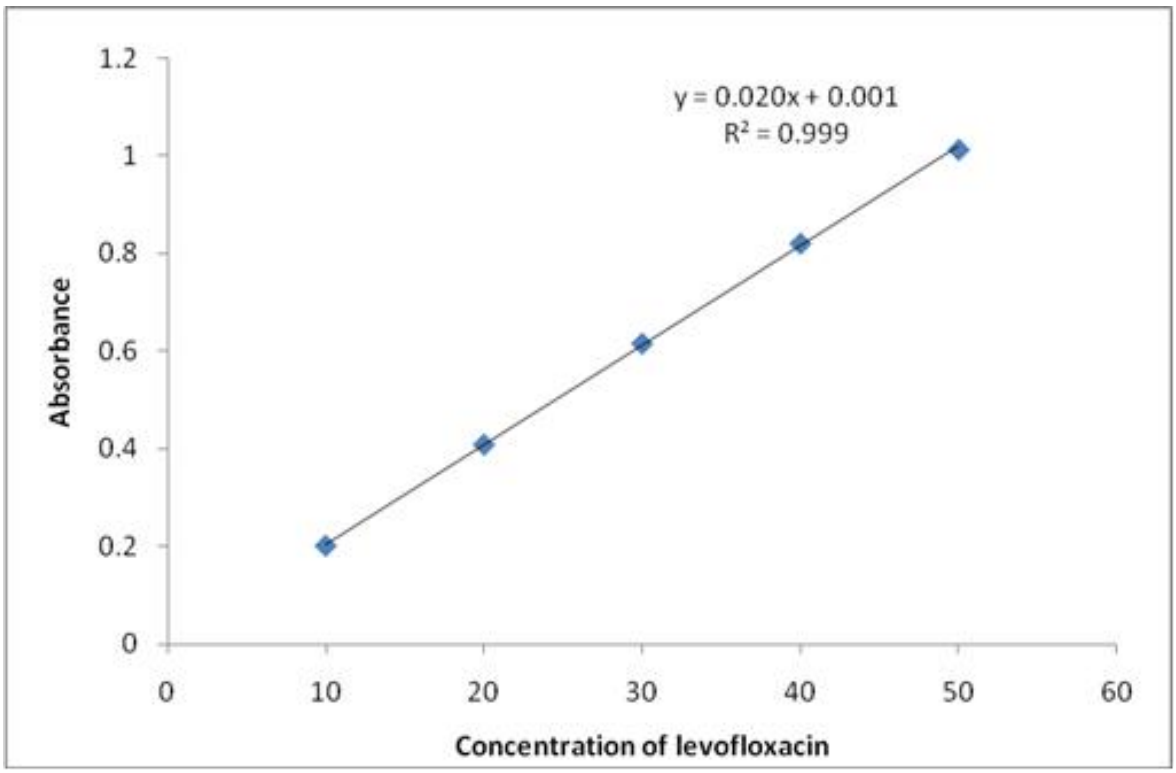

Figure 2: Calibration curve for Levofloxacin

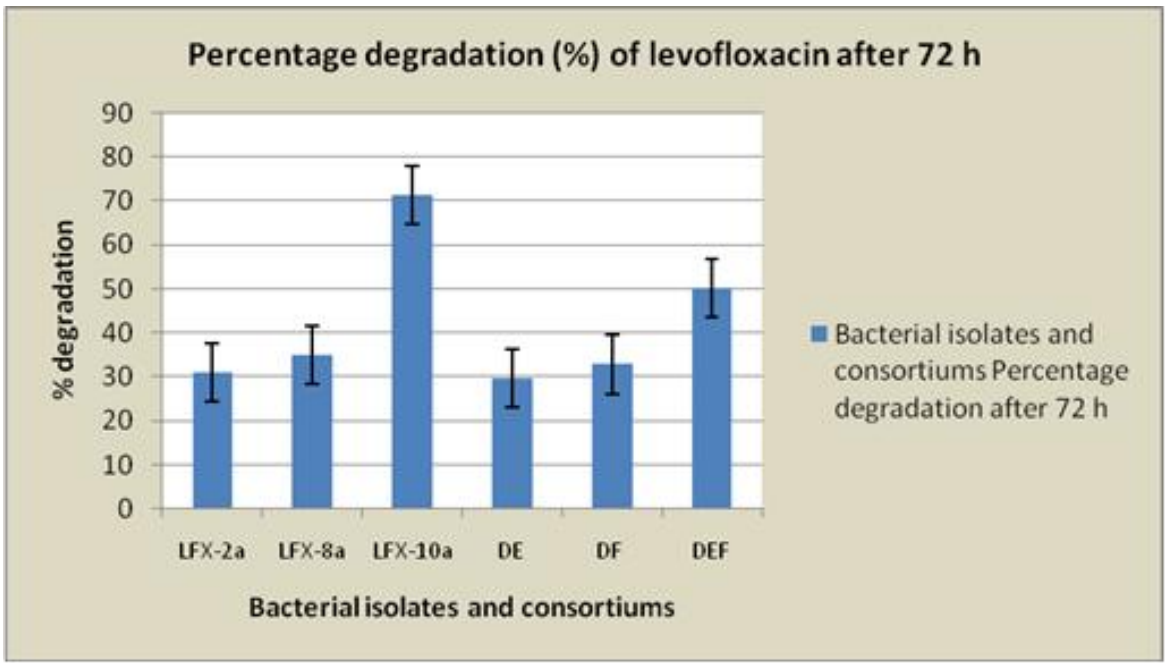

Figure 3: Biodegradation of Levofloxacin by selected bacterial isolates and their consortiums at various incubation periods

Growth study of bacterial isolates at varying concentration of Levofloxacin (LFX)

The growth assessment of bacterial isolates at different concentrations of levofloxacin (LFX) provides valuable insights into the ability of these isolates to tolerate and potentially degrade the antibiotic. The optical density (OD) values measured at 600 nm over a period of 168 hours indicate significant variations in bacterial growth among different isolates and LFX concentrations. Growth pattern of levofloxacin degrading bacterial isolates shown in Table 5.20. After analysis of growth pattern of levofloxacin degrading bacterial isolates it concluded that at 50ppm-500ppm concentration of antibiotic majorly 03 isolates named NFX-2a, NFX-8a and LFX-10a showed good and increasing growth throughout the period of incubation (Figure 1). The results also highlight a general trend wherein bacterial growth increased over time, peaking around 120–144 hours before stabilizing or slightly declining in some cases. This suggests that the bacteria may be metabolizing the antibiotic as a carbon source, but prolonged exposure might lead to stress or nutrient depletion, affecting further growth.

Morphological characterization

Amongst 09 levofloxacin degrading bacterial isolates, 04 were Gram positive and 05 Gram were negative in nature, all of them are rod in shape. Results revealed that only 4 bacterial isolates (LFX-1a, LFX-8a, LFX-8b and LFX-9a) out of 09 were positive for endospore formation while 04 (LFX-2a, LFX-5a, LFX-7a and LFX-10a) showed positive for capsule formation. Only 07 isolates (LFX-1a, LFX-2a, LFX-5a, LFX-7a, LFX-8a, LFX-8b and LFX-9a) were found motile in nature.

Biochemical characterization of levofloxacin degrading bacterial isolates

Results of 09 levofloxacin degrading bacterial isolates revealed that only 04 bacterial isolates were able to tolerate 6.5% of NaCl. All levofloxacin degrading bacterial isolates were able to produce catalase while only 02 of them (LFX-1a and LFX-2a) were able to produce oxidase enzyme and found positive. Additionally, 03 bacterial isolates (LFX-3a, LFX-5a and LFX-9a) for esculin hydrolysis, 03 isolates for amylase production (LFX-1a, LFX-2a and LFX-8b), 07 isolates for nitrate and 02 (LFX-1a and LFX-10a) for urease production were found positive. Results of IMViC revealed that only 02 bacterial isolate (LFX-5a and LFX-7a) showed positive result for indole production, 05 isolates (LFX-1a, LFX-3a, LFX-5a, LFX-8a and LFX-9a) were positive for methyl red, 04 isolates (LFX-8a, LFX-8b, LFX-9a and LFX-10a) positive for VP and 05 isolates (LFX-2a, LFX-3a, LFX-7a, LFX-8b and LFX-10a) positive for citrate utilization.

Quantitative analysis of Levofloxacin degradation by selected bacterial isolates

A total of 03 bacterial isolates named LFX-2a, LFX-8a, LFX-10a and their consortiums DE (LFX-2a+LFX- 8a), DF (LFX-2a+LFX-10a) and DEF (LFX-2a+LFX-8a+LFX-10a) were selected for this study. For this purpose, optimized condition was used for each isolates and for consortium, control condition was used. For the degradation study, calibration curve was prepared with known concentration of antibiotic levofloxacin (Figure 2). Later absorbance of unknown concentration was taken and % degradation was calculated. Samples were taken at 0h and 72h of incubation period for the degradation study.

Bacterial isolate LFX-10a showed maximum % degradation of 71.24% of levofloxacin after 72h followed by DEF (LFX-2a+LFX-8a+LFX-10a) (50.24%), LFX-8a (34.86%), DF (LFX-2a+LFX-10a) (32.84%), LFX-2a (30.92%), DE (LFX-2a+LFX-8a) (29.76%) (Figure 3).

CONCLUSION:

The potential of specific bacterial isolates for the bioremediation of the persistent pharmaceutical contaminant levofloxacin is demonstrated in this study. Nine of the eleven bacterial isolates that were extracted from soil samples using an enrichment procedure showed resistance to levofloxacin concentrations as high as 500 ppm. Superior growth and tolerance were demonstrated by isolates LFX-2a, LFX-8a, and LFX-10a; after 72 hours, LFX-10a had the greatest degradation rate, 71.24%. Significant performance was also shown by the consortium DEF (LFX-2a, LFX-8a, and LFX-10a), which degraded 50.24% of levofloxacin, suggesting possible synergistic effects among these bacteria.

A mixture of rod-shaped, Gram-positive and Gram-negative bacteria were identified by morphological characterisation; seven isolates showed different characteristics, including the development of endospores and capsules and motility. Differential sugar fermentation patterns, nitrate reduction in seven isolates, and catalase synthesis in all isolates were among the metabolic capacities revealed by biochemical tests. These traits probably contribute to the isolates' degradative efficiency. Although extended exposure may cause stress, the observed growth patterns, which peak at 120–144 hours, indicate that these isolates may use levofloxacin as a carbon source. These results highlight the DEF consortium's and LFX-10a's effectiveness as viable options for levofloxacin bioremediation. For useful bioremediation applications, further studies should concentrate on clarifying the enzymatic pathways involved, improving degradation conditions, and assessing the isolates' scalability and environmental safety.

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Conflict of interest:

The authors had no conflict of interest with respect to conduct, authorship, or publication of this research work.

References

1. Wang, J., Wang, S., & Zhao, W. (2019). Biodegradation of levofloxacin by *Bacillus subtilis* isolated from pharmaceutical wastewater. *Environmental Science and Pollution Research*, 26(14), 14287–14295. https://doi.org/10.1007/s11356-019-04676-6
2. Nguyen, P. Y., Carvalho, G., Reis, M. A., & Oehmen, A. (2021). Biodegradation of fluoroquinolone antibiotics and the performance of bacterial consortia in bioreactors. *Bioresource Technology*, 340, 125664. https://doi.org/10.1016/j.biortech.2021.125664
3. Hamdi, H., Hechmi, S., Khelil, M. N., Zoghlami, R. I., Benzarti, S., Mokni-Tlili, S., ... & Jedidi, N. (2020). Biodegradation of pharmaceuticals in wastewater: Performance of bacterial consortia under aerobic and anaerobic conditions. *Journal of Environmental Management*, 275, 111244. https://doi.org/10.1016/j.jenvman.2020.111244
4. Apreja, M., Sharma, A., & Vats, A. (2022). Biodegradation of ciprofloxacin and levofloxacin by consortia of bacteria from pharmaceutical wastewater: Efficiency and microbial community analysis. *Environmental Technology & Innovation*, 27, 102504. https://doi.org/10.1016/j.eti.2022.102504
5. Malik, A. and Jaiswal, R. (2000). Metal resistance in Pseudomonas strains isolated from soil treated with industrial wastewater. *Journal of Microbiology & Biotechnology.* 16, 177-182
6. Bhatia, S. K., & Bhatia, R. K. (2020). Bioremediation of pharmaceutical contaminants from wastewater: Mechanisms and applications. In A. Pandey, S. R. Negi, & P. Binod (Eds.), *Bioremediation of pollutants* (pp. 231–250). Elsevier. https://doi.org/10.1016/B978-0-12-819025-8.00011-0
7. Feng, M., Wang, Z., Dionysiou, D. D., & Sharma, V. K. (2018). Metal-mediated oxidation of fluoroquinolone antibiotics in water: A review. *Chemosphere*, 208, 254–266. https://doi.org/10.1016/j.chemosphere.2018.05.159
8. Kümmerer, K. (2009). Antibiotics in the aquatic environment—A review—Part II. *Chemosphere*, 75(4), 435–441. https://doi.org/10.1016/j.chemosphere.2008.12.006
9. Tahrani, L., Van Loco, J., Ben Mansour, H., & Reyns, T. (2018). Occurrence and fate of fluoroquinolone antibiotics in wastewater treatment plants: A review. *Environmental Science and Pollution Research*, 25(7), 6113–6125. https://doi.org/10.1007/s11356-017-0984-9
10. Tiwari, B., Sellamuthu, B., Ouarda, Y., Drogui, P., Tyagi, R. D., & Buelna, G. (2017). Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. *Bioresource Technology*, 224, 1–12. https://doi.org/10.1016/j.biortech.2016.11.042
11. Xiong, J. Q., Kurade, M. B., & Jeon, B. H. (2018). Can microalgae remove pharmaceutical contaminants from water? *Trends in Biotechnology*, 36(1), 30–44. https://doi.org/10.1016/j.tibtech.2017.09.003