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*Research Article*

# CHARACTERIZATION AND DEGRADATION OF ANTIBIOTIC CEFADROXIL BY SELECTED BACTERIAL ISOLATES

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**Abstract**

**Aim:** Antibiotic contamination in the environment is a growing concern, especially with commonly used drugs like Cefadroxil. This study aimed to isolate and characterize bacterial strains capable of degrading Cefadroxil under optimized laboratory conditions.

**Method:** Soil samples were collected from different locations, and bacterial isolates were enriched and screened for their ability to tolerate and degrade Cefadroxil at various concentrations.

**Results:** Among the isolates, CFR-10 showed the highest degradation efficiency, achieving 50% degradation after 72 hours. Morphological, biochemical, and molecular studies confirmed CFR-10 as closely related to *Enterobacter cloacae*. The bacterial isolates also demonstrated resistance to several heavy metals, indicating their ability to survive under stressful conditions.

**Conclusion:** This study highlights the potential use of indigenous bacterial strains for the bioremediation of Cefadroxil-contaminated environments.

**Keywords:** Cefadroxil degradation, Bioremediation, Enterobacter cloacae, Antibiotic pollution, Heavy metal resistance, Indigenous bacteria

## 1. Introduction

Antibiotic pollution has emerged as one of the most pressing environmental concerns of the 21st century. These life-saving drugs, while essential for human and animal health, are increasingly found in natural ecosystems, primarily due to their overuse, improper disposal, and inadequate removal through conventional wastewater treatment processes. One of the most frequently detected antibiotics in environmental matrices is Cefadroxil, a β-lactam antibiotic belonging to the first-generation cephalosporin class. Used widely to treat respiratory tract infections, urinary tract infections, and skin diseases, cefadroxil functions by inhibiting bacterial cell wall synthesis. However, due to its incomplete metabolism and excretion in an unchanged form, cefadroxil often enters sewage systems and eventually makes its way into rivers, soil, and groundwater sources. The excessive and often indiscriminate use of antibiotics has become one of the leading causes of pharmaceutical contamination in the environment [1,2]. Antibiotics reach the natural ecosystems through various pathways, including pharmaceutical manufacturing effluents, hospital and municipal wastewater discharge, and agricultural runoff resulting from veterinary applications [3,4]. Among the many antibiotics detected, cephalosporins, particularly Cefadroxil, represent a significant group due to their widespread clinical and veterinary use [5,6].

Cefadroxil is a first-generation cephalosporin antibiotic known for its broad-spectrum antibacterial activity against Gram-positive and Gram-negative pathogens [7].

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Its chemical structure includes a β-lactam ring, making it susceptible to hydrolysis under environmental conditions but still stable enough to persist if untreated [8]. Residues of Cefadroxil have been found in surface waters, soils, and even drinking water systems, leading to concerns over their ecological impacts and the promotion of antibiotic resistance genes (ARGs) in microbial communities [9].

The environmental stability and persistence of antibiotics like Cefadroxil pose two major threats. Firstly, they exert selective pressure on environmental microbiota, contributing to the emergence and spread of antibiotic-resistant bacteria (ARB) [10]. Secondly, they disturb natural microbial community dynamics, affecting important ecosystem services such as nutrient cycling and organic matter decomposition [11]. Conventional wastewater treatment plants (WWTPs) often fail to completely remove such pharmaceutical residues, making alternative degradation strategies urgently needed.

In this context, bioremediation using bacteria capable of antibiotic degradation offers a sustainable and efficient solution. Indigenous bacterial strains have adapted to local environmental conditions and are often more effective at degrading xenobiotic compounds like Cefadroxil [12]. Bacteria such as *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Stenotrophomonas* have been frequently reported for their ability to biodegrade various classes of antibiotics, including β-lactams, through enzymatic hydrolysis and oxidation-reduction mechanisms [13].

Environmental factors such as pH and temperature are known to significantly influence bacterial growth and degradation activity [14]. Studies show that optimal degradation generally occurs at neutral or slightly alkaline pH (7–9) and moderate temperatures (30–37°C) [15]. Bacterial strains that also exhibit resistance to heavy metals such as copper, zinc, and chromium are particularly valuable, as antibiotic and heavy metal co-contamination is common in real environmental settings [15].

Molecular tools such as 16S rRNA gene sequencing are essential for the accurate identification of potent bacterial degraders. Phylogenetic analysis using molecular data not only helps confirm taxonomic identity but also aids in understanding evolutionary relationships among bacterial isolates [16,17]. Advances in computational biology have made it possible to construct robust phylogenetic trees, improving the classification and tracking of environmental bacterial strains with bioremediation potential.

Despite the known environmental risks of Cefadroxil, research specifically focusing on its microbial degradation is still limited compared to other antibiotic classes like tetracyclines or sulfonamides [7,8]. Therefore, the isolation, characterization, and evaluation of bacterial isolates capable of degrading Cefadroxil under varying environmental conditions become crucial steps toward developing biotechnological solutions for antibiotic pollution management.

The present study was designed with the objective of isolating indigenous bacterial strains from soil capable of degrading Cefadroxil, optimizing environmental conditions for their growth, and characterizing the isolates both biochemically and molecularly. It aims to contribute to the limited but growing field of antibiotic bioremediation research by identifying potential bacterial candidates for future wastewater treatment and environmental restoration applications.

## 2. Methodology

### 2.1 Sample Collection

A total of sixteen soil samples were collected from diverse sites in Gwalior, Madhya Pradesh, using sterilized polythene bags at a depth of 7 cm. Environmental parameters such as soil temperature and pH were recorded at the time of sampling. Samples were transported and stored at 4°C for further microbiological processing.

### 2.2 Enrichment and Acclimatization of Bacterial Isolates

To enrich cefadroxil-degrading bacteria, 1 g of soil was suspended in 9 mL of sterile distilled water and serially diluted. These dilutions were inoculated into Minimal Salt Medium (MSM) supplemented with 20 µg/mL of cefadroxil as the sole carbon source. Cultures were incubated at 30°C with shaking (150 rpm) for 7 days. Acclimatization was done by gradually increasing cefadroxil concentrations up to 100 µg/mL in four stages, each lasting four days.

### 2.3 Isolation and Purification

Post-acclimatization, the cultures were serially diluted and plated on LB agar. Distinct colonies were isolated based on morphology, purified using the streak plate method, and stored at -20°C in nutrient broth with 15% glycerol.

### 2.4 Growth Studies at Varying Cefadroxil Concentrations

Purified isolates were tested in MSM broth containing increasing cefadroxil concentrations (50 to 500 ppm). Cultures were incubated at 30 ± 2°C, 120 rpm for 168 hours, and bacterial growth was monitored by measuring OD600 at 24-hour intervals using a UV-visible spectrophotometer.

### 2.5 Maximum Tolerance Level (MTL)

Isolates were streaked on LB agar supplemented with cefadroxil at concentrations ranging from 50 to 500 ppm. Plates were incubated at 37°C for 24–48 hours. Growth was visually scored from no growth (-) to maximum (+++), determining each isolate's tolerance level.

### 2.6 Heavy Metal Resistance

Resistance to $ZnSO_4$, $CuSO_4$, $K_2Cr_2O_7$, $CdCl_2$, and $HgCl_2$ (0.1–1.0%) was evaluated using the well diffusion method on LB agar. Zones of inhibition were recorded to assess resistance levels.

**2.7 Morphological and Biochemical Characterization**

Colony morphology and staining (Gram, capsule, endospore) were documented. Biochemical tests (catalase, oxidase, indole, citrate, MR-VP, esculin hydrolysis, carbohydrate fermentation, urease, amylase, nitrate reduction, and salt tolerance) were conducted using standard protocols (Aneja, 2003).

**2.8 Optimization of Environmental Conditions**

Cefadroxil degradation was tested at varying pH levels (3, 5, 7, 9, 11) and temperatures (20°C–50°C) in MSM broth with 50 ppm cefadroxil. OD600 was measured at 24-hour intervals for 72 hours to determine optimal degradation conditions.

**2.9 Quantitative Degradation Assay**

Selected isolates were grown to log phase and inoculated into 50 mL sterile nutrient broth containing 50 mg/L cefadroxil. Cultures were incubated at 37°C, 150 rpm for 7 days. Samples were collected at intervals (0, 1, 3, 5, 7 days), centrifuged, and analyzed via UV-visible spectrophotometry at 260 nm. Antibiotic degradation percentage was calculated using initial ($C_0$) and residual concentrations (Ct).

**2.10 Molecular Identification**

Genomic DNA of potent cefadroxil-degrading isolate (CFR-10) was extracted and 16S rRNA gene (~1500 bp) amplified using F8/1542R primers. PCR products were sequenced and BLAST analyzed to determine phylogenetic affiliation. A phylogenetic tree was constructed using MEGA7 employing the Neighbor-Joining method with 1000 bootstrap replicates.

**2.11 Statistical Analysis**

Data were analyzed using one-way ANOVA in SPSS 16.0. Means and standard deviations were computed in Excel. Correlation analyses between antibiotic concentration and bacterial growth were performed in R (version 4.1.1).

**4. Data Analysis and Results**

**4.1 Enrichment and Isolation of Cefadroxil-Degrading Bacterial Isolates**

A total of 12 bacterial isolates capable of degrading the antibiotic Cefadroxil were successfully obtained through an enrichment culture technique from soil samples collected at various locations. The enrichment was carried out in Minimal Salt Medium (MSM), where Cefadroxil was provided as the sole carbon and energy source. A gradual increase in turbidity and a noticeable color change in the medium, compared to uninoculated controls, indicated bacterial growth and potential antibiotic degradation. After multiple subcultures with increasing concentrations of Cefadroxil, the final enriched cultures were serially diluted (up to 1:1000), spread on MSM agar plates containing Cefadroxil, and incubated at 37°C for 48 hours. Morphologically distinct colonies were selected, purified, and maintained on MSM agar plates supplemented with Cefadroxil. These 12 isolates were preserved in pure culture at 4°C and were further analyzed for their growth responses at varying concentrations of Cefadroxil to evaluate their degradation potential.

| S.No. | Sample code | Sample collection Area | Cefadroxin degrading bacteria |
|---|---|---|---|
| 1. | SS-1 | Near Ranbaxy Laboratories, Malanpur | CFR-1 |
| 2. | SS-2 | Ddumping site, DRDO, Gwalior | |
| 3. | SS-3 | Dumping Site, Sharma Farm, Banmore | CFR-2 |
| 4. | SS-4 | Dumping Site, Hazira, Gwalior | CFR-3 |
| 5. | SS-5 | Hurawali Churaha, Sirol Road, Gwalior | CFR-4 |
| 6. | SS-6 | Near Morar Anaj Mandi, Morar, | CFR-5 |

| S.No. | Sample code | Sample collection Area | Cefadroxin degrading bacteria |
|---|---|---|---|
| | | Gwalior | |
| 7. | SS-7 | Oppsit DD mall, Shinde ki Chhavni, Gwalior | CFR-6 |
| 8. | SS-8 | Dumping site, Near Chhatri Mandi, Gwalior | CFR-7 |
| 9. | SS-9 | ShreeRam Motor and repairs, Transport Nagar, Gwalior | CFR-8 |
| 10. | SS-10 | NavChetan Automobiles, Bahodapur, Gwalior | CFR-9 |
| 11. | SS-11 | Shameem automobilesm and repair Shop, Thatipur | CFR-10 |
| 12. | SS-12 | Dumping site, Near Sabji Mandi, Laxmi Ganj, Gwalior | |
| 13. | SS-13 | BCG Petrochemical Plant, Banmore | CFR-11 |
| 14. | SS-14 | Loco car wash, Hazeera, Gwalior | CFR-12 |
| **Total number of antibiotic degrading bacterial isolates** | | | 12 |

### 4.2 Biological Degradation of Cefadroxil

The biological degradation of Cefadroxil by selected bacterial isolates was evaluated by studying their growth at different antibiotic concentrations ranging from 50 ppm to 500 ppm. It was observed that the majority of isolates exhibited good growth at lower concentrations, particularly at 50 ppm, indicating their ability to utilize or tolerate Cefadroxil under mild stress conditions. At higher concentrations, growth generally declined; however, a few isolates showed notable adaptation. Among them, one isolate demonstrated significant growth even at 500 ppm concentration after 24 hours of incubation, suggesting strong resistance and potential for efficient degradation of Cefadroxil at elevated levels. Overall, the findings confirmed the ability of the selected bacterial strains to survive and possibly degrade Cefadroxil across a range of concentrations.

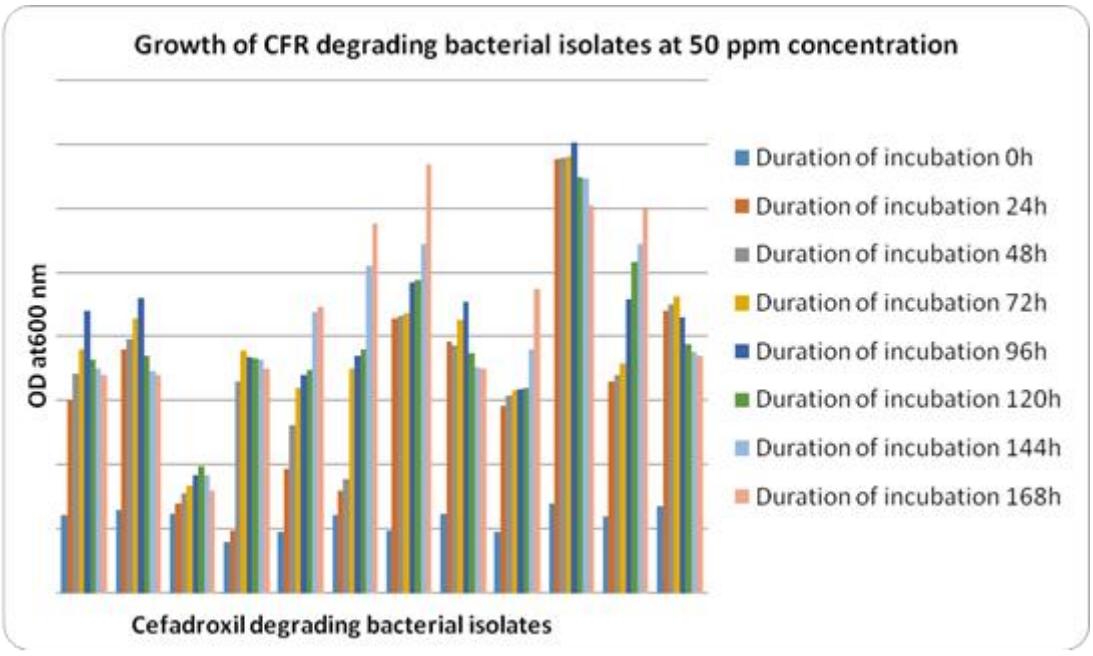


**Figure 3: Relationship between growth and incubation period of Cefadroxil degrading bacterial isolate at 50 ppm concentration of Cefadroxil (OD at 600nm)**

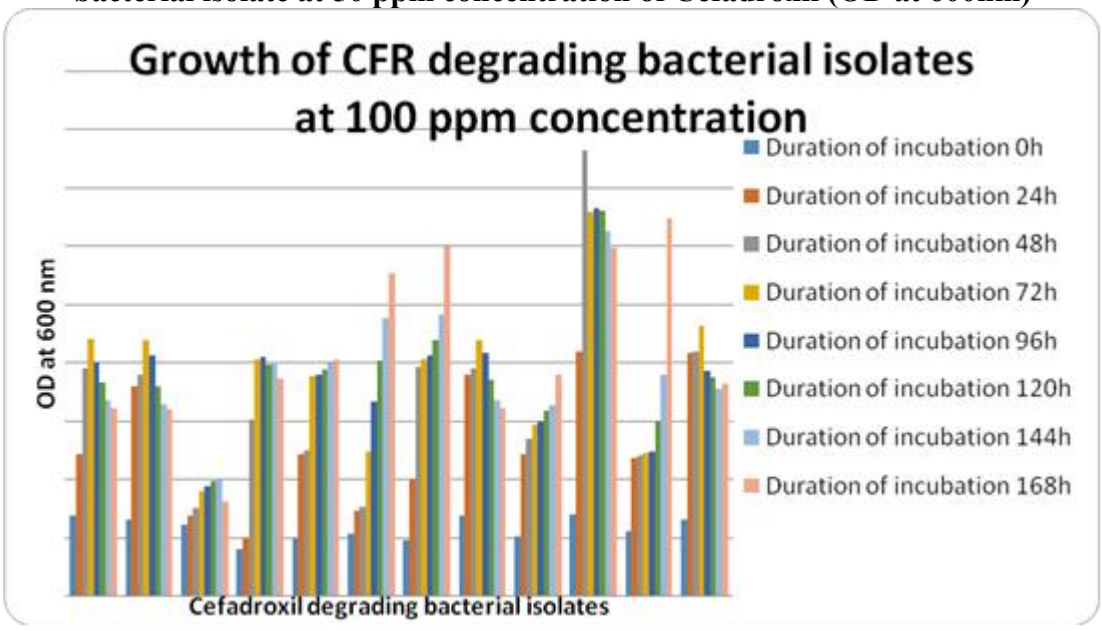


**Figure 4: Relationship between growth and duration of incubation of cefadroxil degrading bacterial isolate at 100 ppm concentration of cefadroxil (OD at 600nm)**

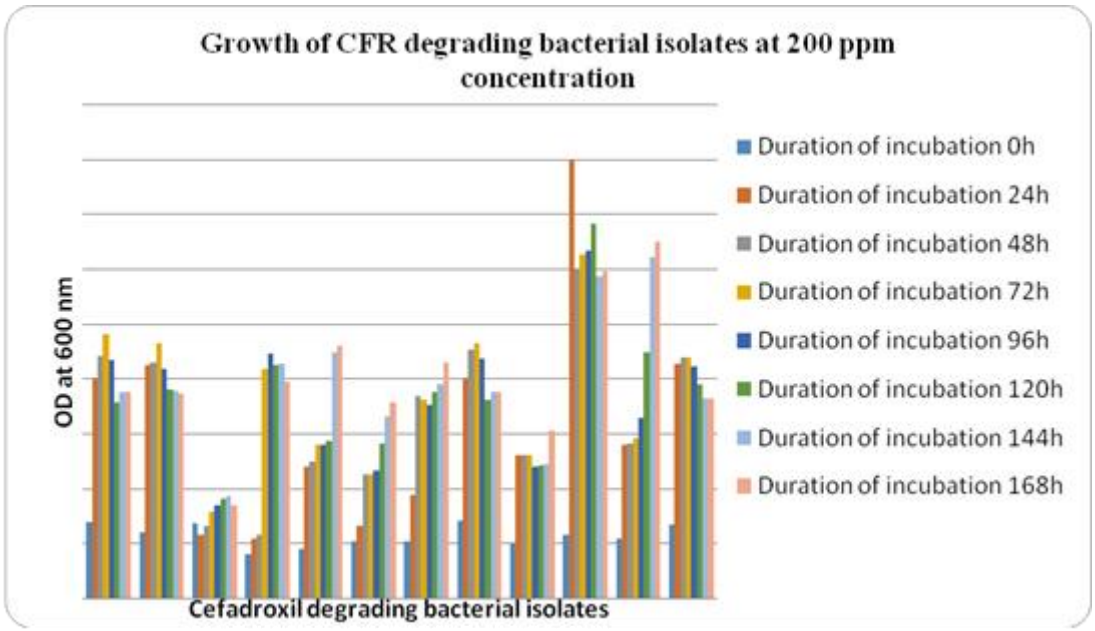


**Figure 5: Relationship between growth and duration of incubation of Cefadroxil degrading bacterial isolate at 200 ppm concentration of Cefadroxil (OD at 600nm)**

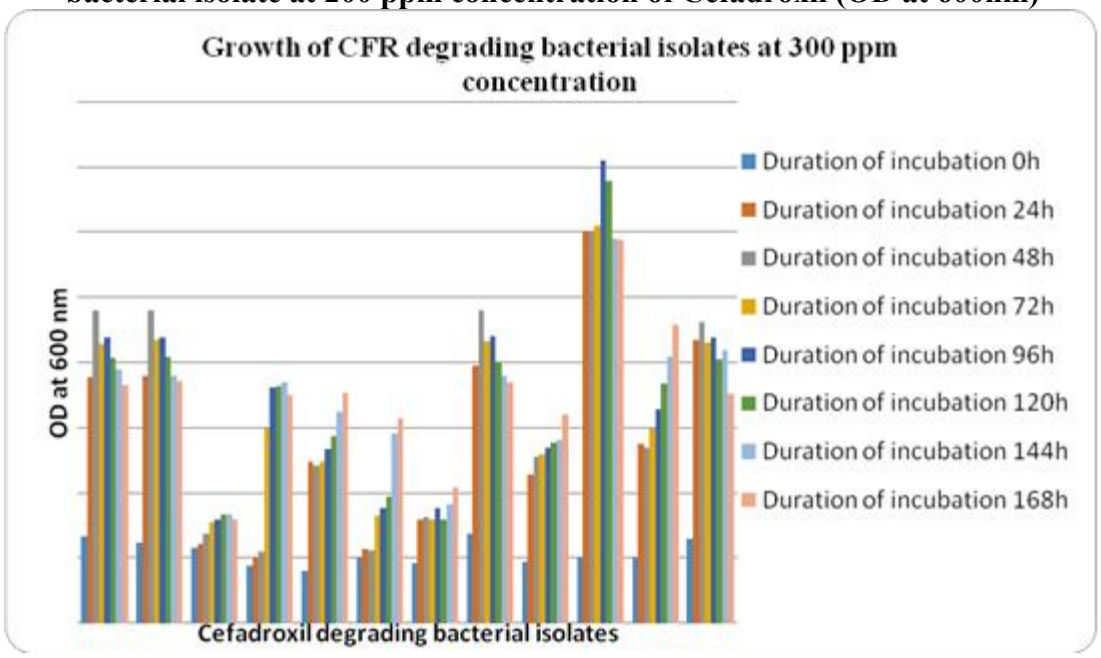


**Figure 6: Relationship between growth and duration of incubation of Cefadroxil degrading bacterial isolate at 300 ppm concentration of Cefadroxil (OD at 600nm)**

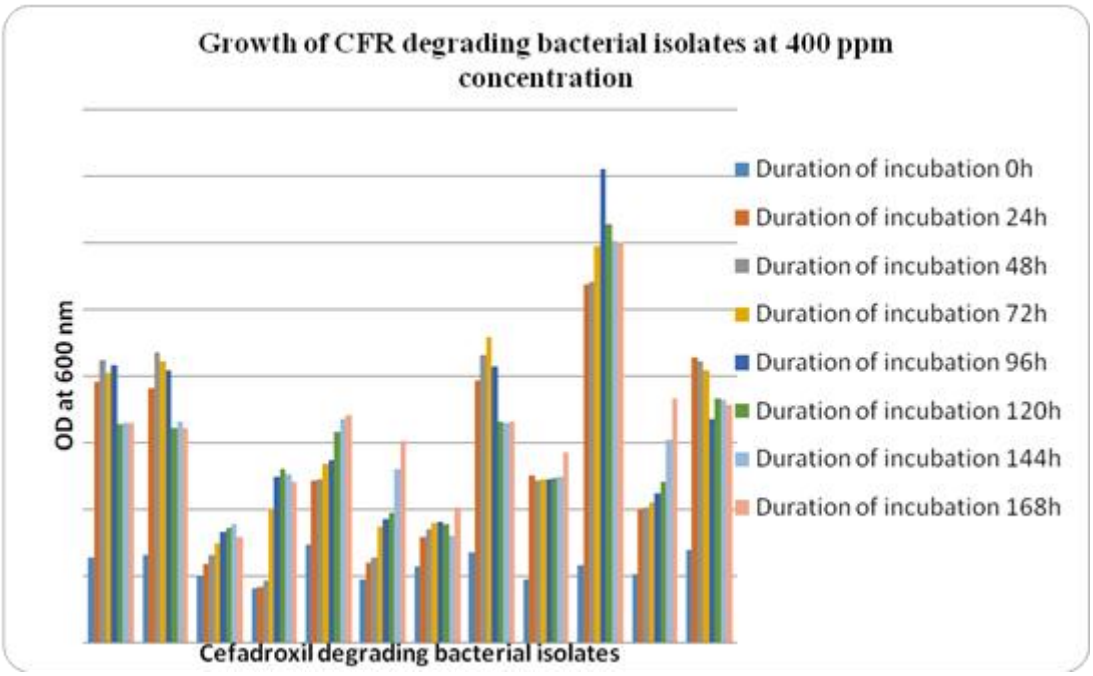


**Figure 7: Relationship between growth and duration of incubation of Cefadroxil degrading bacterial isolate at 400 ppm concentration of Cefadroxil (OD at 600nm)**

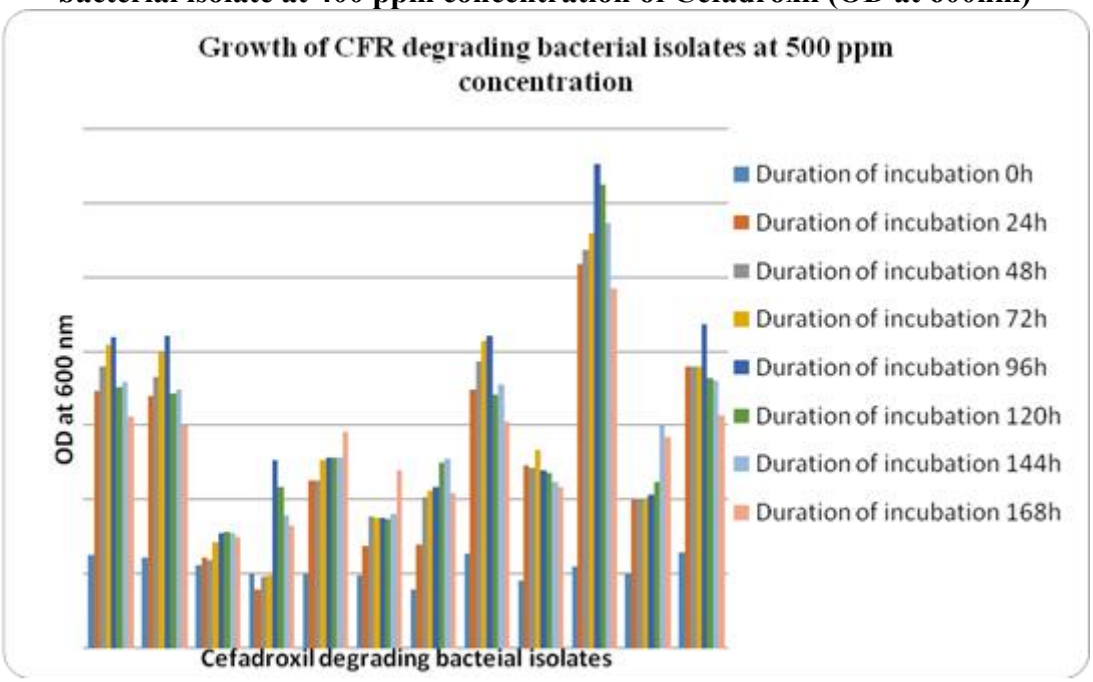


**Figure 8: Relationship between growth and duration of incubation of Cefadroxil degrading bacterial isolate at 500 ppm concentration of Cefadroxil (OD at 600nm)**

### 4.3 Screening of Bacterial Isolates for Maximum Tolerance Level (MTL) of Cefadroxil

The Maximum Tolerance Level (MTL) of Cefadroxil-degrading bacterial isolates was determined by evaluating their growth across increasing concentrations of Cefadroxil (50–500 ppm) using a visual comparison method. Among the twelve isolates tested, isolate CFR-10 exhibited maximum growth at all tested concentrations, indicating strong tolerance towards high levels of Cefadroxil. Other isolates such as CFR-1 and CFR-12 displayed moderate growth up to 500 ppm, whereas CFR-3 showed minimal growth only up to 300 ppm and no growth at higher concentrations. Overall, out of twelve isolates, eleven were able to tolerate up to 500 ppm of Cefadroxil, suggesting that the majority of the selected strains possessed a high tolerance and potential for effective degradation of Cefadroxil even under antibiotic stress.

**Table 3: Maximum Tolerance Level (MTL) of Cefadroxil degrading bacterial isolates at six successive concentrations of Cefadroxil**

| Bacterial isolates | Bacterial growth at varying concentration of Cefadroxil (CFR) | | | | | |
|---|---|---|---|---|---|---|
| | 50 ppm | 100 ppm | 200ppm | 300ppm | 400ppm | 500ppm |
| CFR-1 | ++ | ++ | ++ | ++ | ++ | ++ |
| CFR-2 | ++ | ++ | ++ | ++ | ++ | ++ |
| CFR-3 | + | + | + | + | - | - |
| CFR-4 | ++ | ++ | + | + | + | + |
| CFR-5 | ++ | ++ | ++ | ++ | ++ | + |
| CFR-6 | +++ | +++ | +++ | ++ | ++ | + |
| CFR-7 | +++ | +++ | +++ | ++ | ++ | ++ |
| CFR-8 | ++ | ++ | ++ | ++ | ++ | ++ |
| CFR-9 | ++ | ++ | + | + | + | + |
| CFR-10 | +++ | +++ | +++ | +++ | +++ | +++ |
| CFR-11 | +++ | +++ | +++ | ++ | ++ | + |
| CFR-12 | ++ | ++ | ++ | ++ | ++ | ++ |

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

### 4.4 Heavy Metal Resistance of Cefadroxil-Degrading Bacterial Isolates

The heavy metal resistance ability of the Cefadroxil-degrading bacterial isolates was assessed against five heavy metals (zinc, copper, chromium, cadmium, and mercury) at concentrations of 0.1%, 0.5%, and 1.0%. At 0.1% concentration, the majority of isolates demonstrated resistance to zinc and copper, with lower resistance observed for chromium and cadmium, and no resistance against mercury. Isolates CFR-2, CFR-7, and CFR-10 showed resistance to multiple heavy metals, including copper, zinc, chromium, and cadmium. At 0.5% concentration, a similar pattern was observed where copper and zinc resistance remained predominant, while resistance to chromium declined and no isolates resisted cadmium or mercury. At the highest tested concentration of 1.0%, resistance was highest against copper and zinc, minimal for chromium, and absent for cadmium and mercury. Notably, isolate CFR-7 consistently demonstrated resistance to zinc, copper, and chromium even at higher concentrations, indicating strong multi-metal resistance capabilities alongside its antibiotic tolerance.

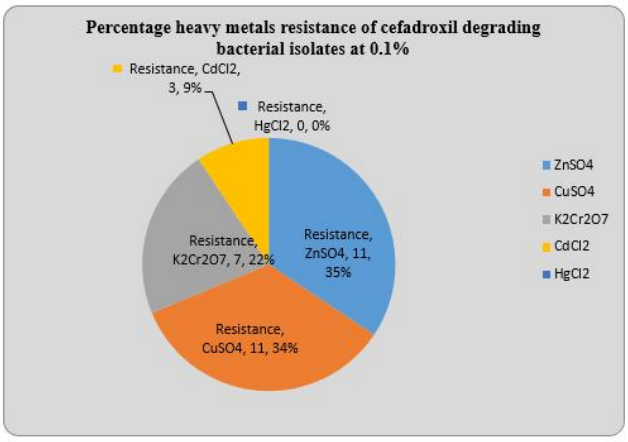


**Figure 9: Percentage heavy metals resistance of the Cefadroxil degrading bacterial isolates at 0.1 % metal concentration**

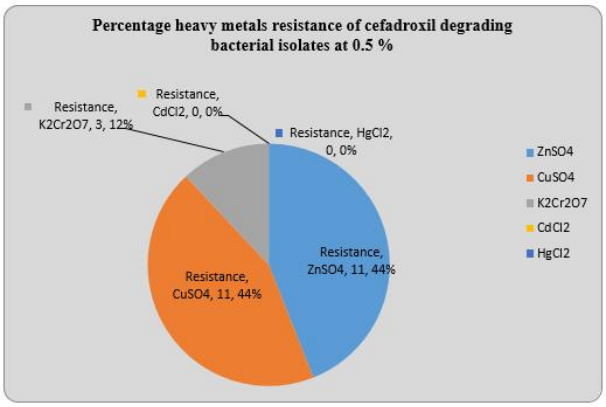


**Figure 10: Percentage heavy metals resistance of the Cefadroxil degrading bacterial isolates at 0.5 % metal concentration**

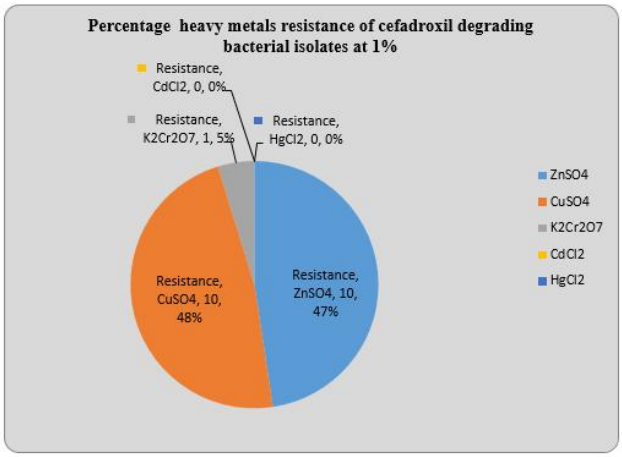


**Figure 11: Percentage heavy metals resistance of the Cefadroxil degrading bacterial isolates at 1 % metal concentration**

### 4.5 Morphological and Biochemical Characterization of Cefadroxil-Degrading Bacterial Isolates

#### 4.5.1 Morphological Characterization of Cefadroxil-Degrading Bacterial Isolates

The morphological and biochemical characterization of the Cefadroxil-degrading bacterial isolates was carried out to study their basic cellular and structural properties. Among the twelve isolates analyzed, eight were found to be Gram-positive and four were Gram-negative, with most exhibiting a bacilli (rod-shaped) morphology. Endospore staining revealed that only two isolates, CFR-9 and CFR-11, were positive for endospore formation, indicating the presence of spore-forming capabilities under stress conditions. None of the cefadroxil-degrading isolates tested positive for capsule formation. Regarding motility, four isolates (CFR-1, CFR-5, CFR-6, and CFR-7) were found to be motile, suggesting potential advantages in colonization and degradation processes. A noticeable diversity in colony morphology, including variations in size, shape, form, and margin, was observed among the isolates. These morphological characteristics helped in differentiating and preliminarily identifying the bacterial strains capable of degrading Cefadroxil.

**Table No. 4 Morphological characterization of Cefadroxil (CFR) degrading bacterial isolates**

| S.No | Antibiotic degrading bacterial isolates | Colony characteristics | Gram's nature, shape and arrangement | Endospore staining | Capsule staining | Motility test |
|---|---|---|---|---|---|---|
| 1. | CFR-1 | Medium, circular, entire, smooth, white | Gram positive, rod | - | - | Motile |

| S.No | Antibiotic degrading bacterial isolates | Colony characteristics | Gram's nature, shape and arrangement | Endospore staining | Capsule staining | Motility test |
|---|---|---|---|---|---|---|
| 2. | CFR-2 | White, medium, entire, moist, opaque, | Gram positive, Rod | - | - | Non-motile |
| 3. | CFR-3 | White, medium, wavy, moist, opaque, | Gram positive, Cocci | - | - | Non-motile |
| 4. | CFR-4 | White, punctiform, entire, moist, transparent | Gram negative Rod | - | - | Non-motile |
| 5. | CFR-5 | Large, circular, undulate, smooth, white | Gram negative, Rod | - | + | Motile |
| 6. | CFR-6 | Large, irregular, undulate, glistening, cream | Gram negative, Short rod | - | + | Motile |
| 7. | CFR-7 | Medium, circular, entire, smooth, yellow | Gram negative, Rod | - | - | Motile |
| 8. | CFR-8 | Medium, circular, entire, smooth, cream | Gram positive Cocci | - | - | Non-Motile |
| 9. | CFR-9 | Greenish, medium, wavy, moist and raised | Gram Positive, Rod | + | - | Non-Motile |
| 10. | CFR-10 | White, small, entire, moist, opaque, raised | Gram Positive, Cocci | - | - | Non-Motile |
| 11. | CFR-11 | Cream, large, entire, moist, opaque | Gram Positive, Rod | + | - | Non-Motile |
| 12. | CFR-12 | Large, circular, entire, undulate transparent | Gram Positive, cocci | - | - | Non-Motile |

### 4.5.2 Biochemical Characterization of Cefadroxil-Degrading Bacterial Isolates

The biochemical characterization of Cefadroxil-degrading bacterial isolates revealed significant diversity in their metabolic activities. Among the nine isolates tested, five were able to tolerate 6.5% NaCl concentration, and eight showed positive catalase activity, indicating their aerobic nature. Only one isolate, CFR-8, exhibited oxidase activity. Esculin hydrolysis was positive in three isolates (CFR-5, CFR-6, and CFR-8), while two isolates (CFR-2 and CFR-11) showed amylase production. Most isolates demonstrated the ability to reduce nitrate, except CFR-1, and three isolates (CFR-7, CFR-10, and CFR-12) were found positive for urease production. IMViC test results showed that two isolates (CFR-6 and CFR-12) were positive for indole production, two (CFR-6 and CFR-10) for methyl red, five isolates for Voges-Proskauer, and seven isolates for citrate utilization. In carbohydrate fermentation tests, selected isolates were able to ferment sugars like dextrose, sucrose, fructose, lactose, mannose, inulin, and inositol with varying patterns, whereas none could ferment raffinose and rhamnose. The overall biochemical profile suggested that the isolates belonged to genera such as *Bacillus*, *Micrococcus*, *Enterococcus*, *Pseudomonas*, and related groups as per Bergey's Manual of Determinative Bacteriology.

## Table No. 5 Biochemical characterization of Cefadroxil (CFR) degrading bacterial isolates

| Antibiotic degrading bacterial isolates | Growth in 6.5% NaCl | Catalase | Oxidase | Esculin hydrolysis | Indole | MR | VP | Citrate utilization | Nitrate reduction | Urease production | Amylase | Tentative Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CFR-1** | + | + | - | - | - | - | - | + | - | - | - | *Brevibacterium casei* |
| **CFR-2** | - | + | - | - | - | - | + | + | + | - | + | *Bacillus* |

| Antibiotic degrading bacterial isolates | Growth in 6.5% NaCl | Catalase | Oxidase | Esculin hydrolysis | Indole | MR | VP | Citrate utilization | Nitrate reduction | Urease production | Amylase | Tentative Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | *cereus* |
| **CFR-5** | + | + | - | + | - | - | + | + | + | - | - | *Enterobacter sp.* |
| **CFR-6** | - | + | - | + | + | + | - | - | + | - | - | *Escherichia coli* |
| **CFR-7** | + | + | - | - | - | - | + | + | + | + | - | *Enterobacter sp.* |
| **CFR-8** | + | + | + | + | - | - | - | - | + | - | - | *Micrococcus sp.* |
| **CFR-10** | + | + | - | - | - | + | + | + | + | + | - | *Staphylococcus aureaus* |
| **CFR-11** | - | + | - | - | - | - | + | + | + | - | + | *Bacillus cereus* |
| **CFR-12** | - | - | - | - | + | - | - | + | + | + | - | *Corynebacterium* Xerosis |

## 4.6 Characterization of Environmental Conditions for the Optimal Growth of Cefadroxil-Degrading Bacteria

### 4.6.1 Effect of pH on the Growth of Cefadroxil-Degrading Bacteria

The influence of different pH levels (3, 5, 7, 9, and 11) on the growth of nine selected Cefadroxil-degrading bacterial isolates was assessed over a 72-hour incubation period in the presence of 50 ppm Cefadroxil. The results indicated that bacterial growth varied significantly with pH. Maximum growth for most isolates was observed at neutral pH (7), particularly for isolates CFR-2, CFR-7, and CFR-12. Some isolates, such as CFR-1, CFR-5, CFR-6, and CFR-10, demonstrated better growth under slightly alkaline conditions at pH 9, while isolates CFR-8 and CFR-11 showed comparatively good growth even at highly alkaline pH 11. In contrast, growth was generally reduced under highly acidic conditions (pH 3 and pH 5). These findings suggest that neutral to slightly alkaline conditions favor the optimal growth of most Cefadroxil-degrading bacteria.

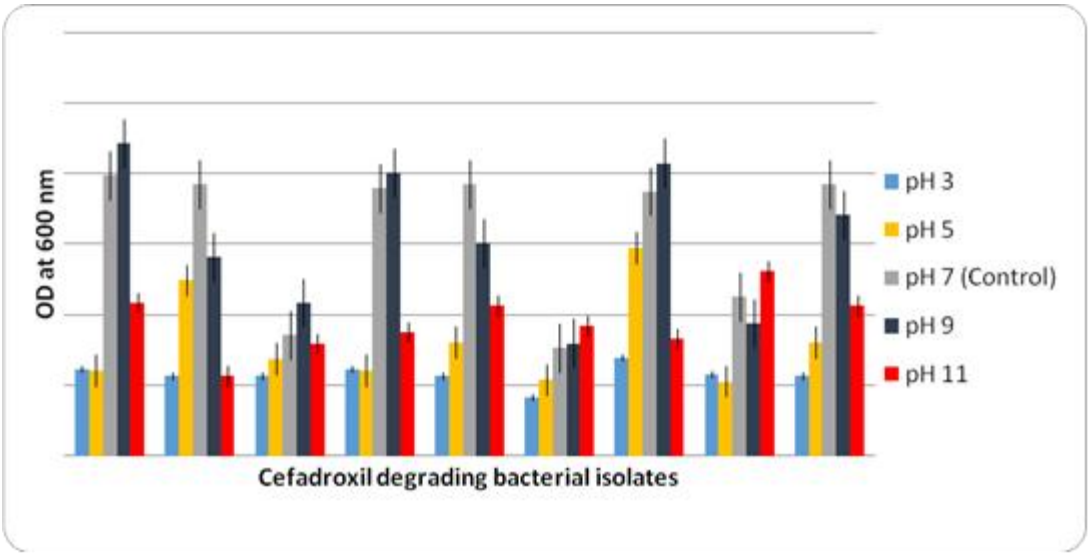

**Figure 12: Growth of Cefadroxil degrading bacterial isolates at different pH, at 50ppm Cefadroxil concentration, after 72h of incubation period.**

### 4.6.2 Effect of Temperature on the Growth of Cefadroxil-Degrading Bacteria

The growth response of nine selected Cefadroxil-degrading bacterial isolates was assessed at different temperatures (20°C, 30°C, 37°C, 40°C, and 50°C) over a 72-hour incubation period in the presence of 50 ppm Cefadroxil. The results indicated that most isolates exhibited optimal growth at moderate temperatures, with maximum growth recorded at 37°C for isolates CFR-6, CFR-7, CFR-8, and CFR-10. Three isolates, CFR-2, CFR-5, and CFR-11, showed highest growth at 30°C, while CFR-1 and CFR-12 demonstrated better growth at 40°C. None of the isolates showed maximum growth at the extreme temperatures of 20°C or 50°C. These findings highlight that the optimal temperature range for the efficient growth of Cefadroxil-degrading bacterial isolates lies between 30°C and 40°C, with 37°C being the most favorable.

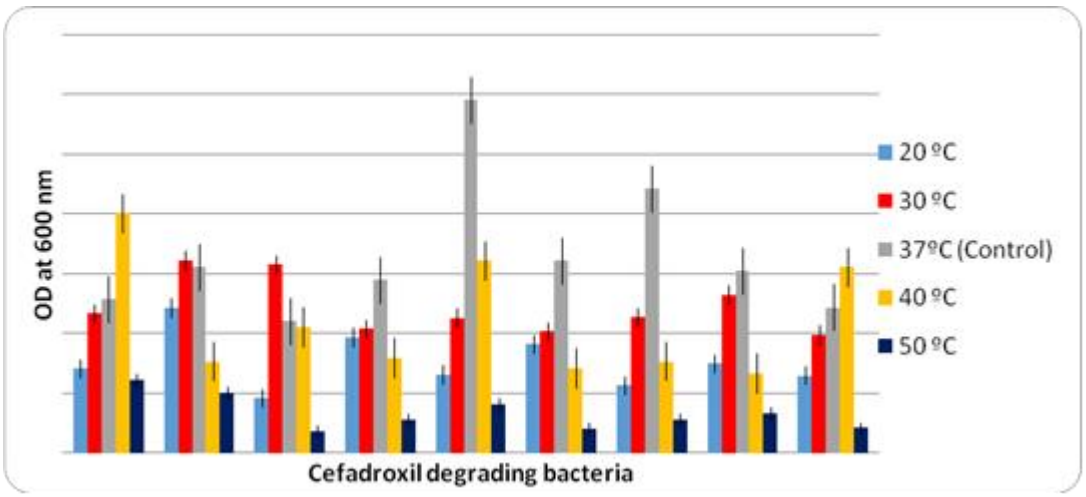


**Figure 7: Growth of Cefadroxil degrading bacterial isolates at different temperature, at 50ppm Cefadroxil concentration, after 72h of incubation period**

### 4.8 Quantitative Analysis of Cefadroxil Degradation by Selected Bacterial Isolates

Quantitative degradation of Cefadroxil by two selected bacterial isolates, CFR-7 and CFR-10, was analyzed under optimized conditions. Calibration curves were prepared using known concentrations of Cefadroxil to determine the antibiotic concentration in degraded samples based on absorbance measurements. The degradation study was performed over an incubation period of 0, 24, 48, and 72 hours. The results revealed that isolate CFR-10 exhibited the highest degradation efficiency, achieving 50% degradation of Cefadroxil after 72 hours, whereas isolate CFR-7 demonstrated 9% degradation over the same period. These findings indicate that CFR-10 possesses a significantly greater ability to degrade Cefadroxil compared to CFR-7 under the given experimental conditions.

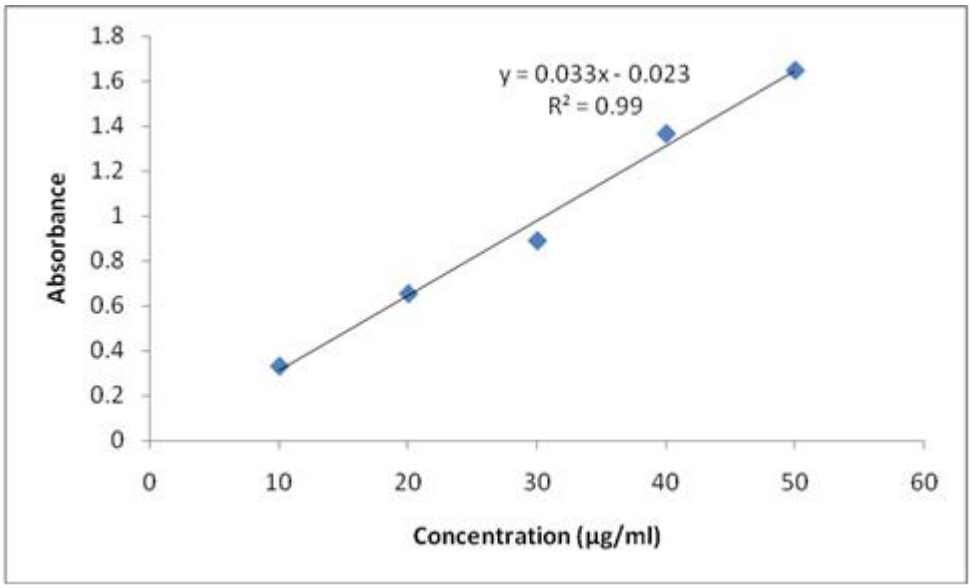


**Figure 8: Calibration curve for Cefadroxil**

### 4.9 Molecular Identification of Cefadroxil-Degrading Bacterial Isolates

The molecular identification of the potent Cefadroxil-degrading bacterial isolate CFR-10 was performed through 16S rRNA gene sequencing. Genomic DNA was extracted and amplified using universal primers F8 (forward) and 1542R (reverse), yielding an amplicon of approximately 1500 bp. The obtained partial 16S rRNA sequence was subjected to BLAST analysis against the NCBI GenBank database. The isolate CFR-10 showed 97.58% sequence similarity with *Enterobacter cloacae* strain DSM 30054, indicating its close phylogenetic relationship to this species. Further phylogenetic analysis was carried out using MEGA 11 software by constructing a Neighbor-Joining tree with 1000 bootstrap replicates, confirming the evolutionary placement of the isolate within the *Enterobacter* genus.

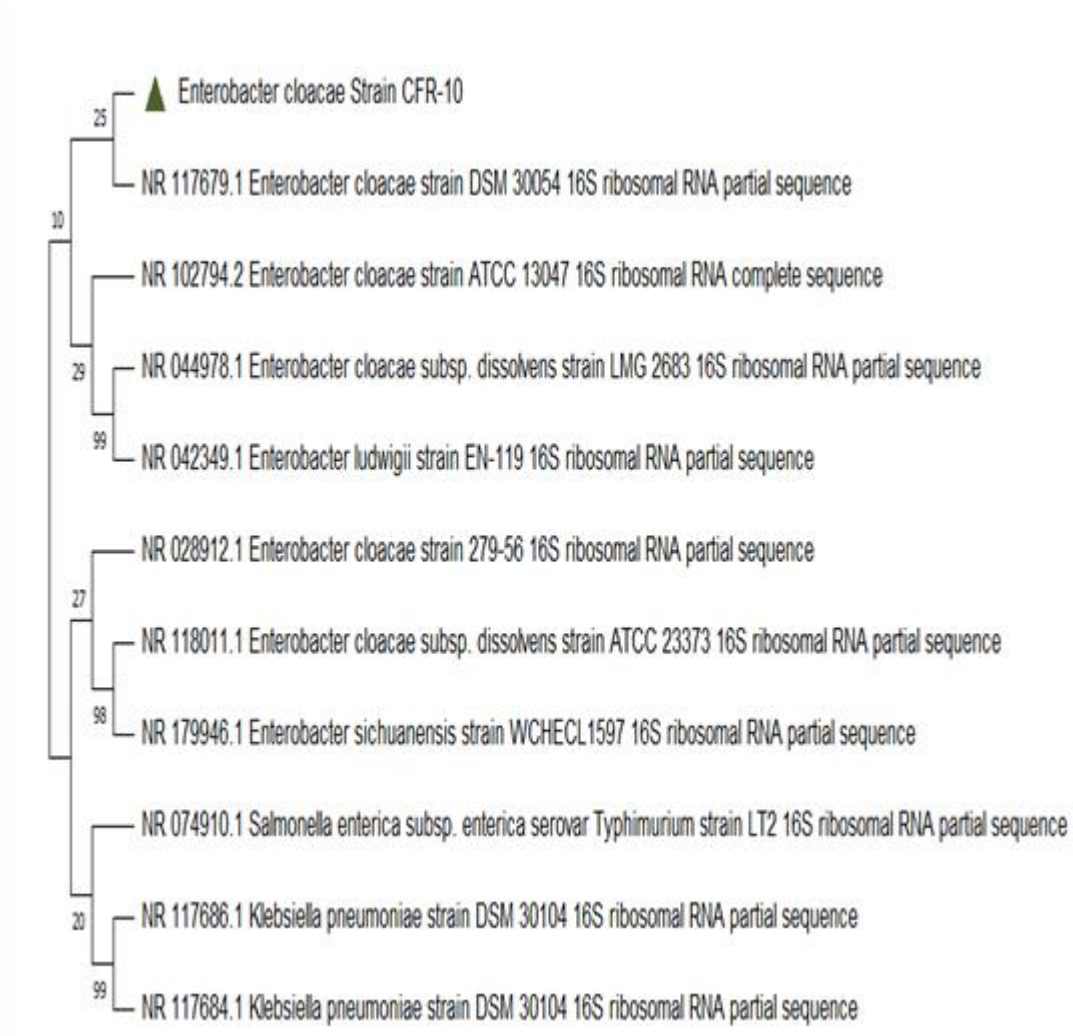


**Figure 9 : Phylogenetic tree of *Enterobacteria cloacae* strain CFR-10 constructed by neighbor joining method using MEGA**

# 5. Conclusion

## 5. Conclusion

The present study successfully demonstrated the potential of indigenous bacterial isolates for the biodegradation of the antibiotic Cefadroxil under optimized laboratory conditions. A total of 12 bacterial strains capable of utilizing Cefadroxil as the sole carbon source were isolated from various environmental sites using the enrichment culture technique. Among these, isolate CFR-10 emerged as the most efficient degrader, exhibiting maximum growth across a wide range of antibiotic concentrations and achieving 50% degradation within 72 hours. The strain also demonstrated significant tolerance to multiple heavy metals, suggesting its robustness under environmental stress. Morphological and biochemical characterization revealed diverse physiological traits among the isolates, while molecular identification confirmed CFR-10 as closely related to *Enterobacter cloacae*. Furthermore, optimization of pH and temperature conditions indicated that neutral to slightly alkaline pH (7–9) and moderate temperatures (30–37°C) are most favorable for bacterial growth and degradation activity. These findings underline the promising role of native bacterial strains such as CFR-10 in the bioremediation of pharmaceutical contaminants like Cefadroxil from polluted environments, contributing toward eco-friendly and sustainable wastewater treatment solutions.

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