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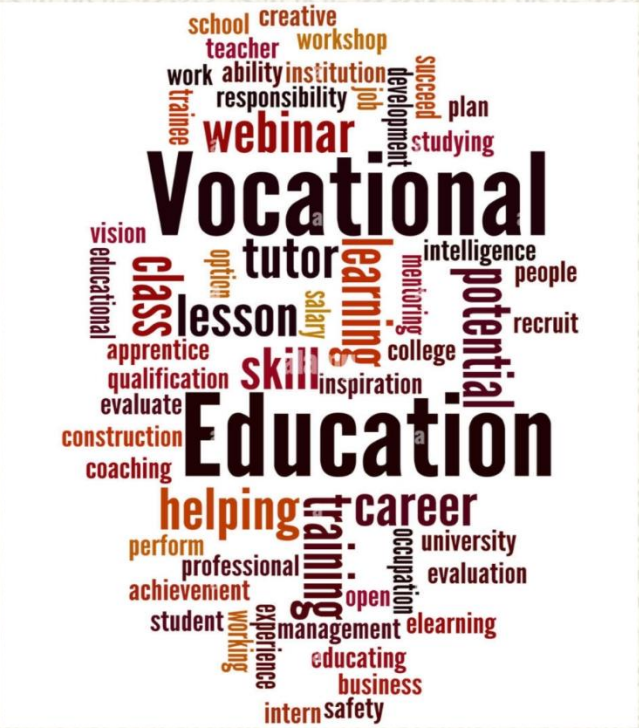

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Comparative Biodegradation of 2,4-Dichlorophenoxyacetic Acid by *Cupriavidus campinensis* and *Achromobacter xylosoxidans* Isolated from Rice Cultivated Soils in Kura, Kano State, Nigeria

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Abstract

This study presents a comparative evaluation of two indigenous bacterial strains, Cupriavidus campinensis and Achromobacter xylosoxidans, isolated from rice cultivated soils in Kura Local Government Area, Kano State, Nigeria, for their ability to degrade 2,4-dichlorophenoxyacetic acid (2,4-D). Both isolates were previously identified and characterized using morphological, biochemical, and molecular techniques. Their biodegradation efficiency was assessed under varying environmental conditions including pH, temperature, substrate concentration, inoculum size, and incubation time. Results revealed that both isolates effectively utilized 2,4-D as a sole carbon and energy source. Achromobacter xylosoxidans exhibited slightly higher degradation efficiency (95.38%) compared to Cupriavidus campinensis (94.69%) under optimal conditions. While both organisms showed optimal degradation at pH 7–7.5 and temperature of 40°C respectively, variations were observed in inoculum size and incubation time requirements. The findings demonstrate that both bacterial strains possess strong bioremediation potential, with Achromobacter xylosoxidans showing marginally superior performance. This comparative study highlights the importance of selecting efficient indigenous microorganisms for sustainable remediation of herbicide-contaminated environments.

Introduction

The rapid intensification of agricultural practices aimed at meeting the increasing global food demand has led to the widespread application of agrochemicals, particularly herbicides, for weed control and crop yield improvement. Among these, 2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most extensively used selective herbicides worldwide due to its effectiveness, affordability, and broad-spectrum activity against dicotyledonous weeds (Jahun *et al*., 2023). Since its introduction in the 1940s, 2,4-D has remained a dominant component in herbicide formulations, accounting for a significant proportion of global pesticide usage (Zhang *et al*., 2023; Wang *et al*., 2024).

Despite its agricultural importance, the persistent application of 2,4-D has resulted in widespread environmental contamination. Due to its high solubility and mobility, 2,4-D readily leaches into soil and aquatic ecosystems, thereby contaminating groundwater and surface water bodies (Jahun *et al*., 2022). Studies have reported the presence of 2,4-D residues in air, water, and biological systems, including human tissues, especially in agricultural communities (Cycoń *et al*., 2021; Patel *et al.,* 2024). Exposure to 2,4-D has been associated with several adverse health effects, including carcinogenicity, neurotoxicity, endocrine disruption, and respiratory complications. Furthermore, the compound has been classified as a moderately hazardous herbicide by international health organizations, emphasizing the need for effective remediation strategies (Kumar *et al*., 2021).

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In addition to human health concerns, the accumulation of 2,4-D in soil negatively impacts soil microbial diversity and fertility, thereby affecting long-term agricultural sustainability. The persistence and degradation rate of 2,4-D in the environment are influenced by several physicochemical factors, including pH, temperature, moisture, and microbial activity (Jahun *et al*., 2024). Conventional remediation methods, such as chemical oxidation and physical removal techniques, are often inefficient, expensive, and may generate secondary pollutants, further complicating environmental management (Sharma *et al*., 2022; Gaur *et al*., 2022).

Consequently, bioremediation has emerged as a promising, eco-friendly, and cost-effective approach for the detoxification of herbicide-contaminated environments. Bioremediation exploits the metabolic capabilities of microorganisms to degrade toxic compounds into less harmful products. Numerous studies have demonstrated that certain bacteria can utilize 2,4-D as a sole carbon and energy source, thereby facilitating its mineralization (Chen *et al*., 2024; Benti *et al*., 2025). The degradation process is typically mediated by specific enzymatic pathways, including dechlorination and oxidative cleavage, often associated with functional genes such as tfdA, which play a crucial role in the initial breakdown of the herbicide (Jahun *et al*., 2024).

A wide range of bacterial genera have been reported to possess the ability to degrade 2,4-D, including Pseudomonas, Alcaligenes, Achromobacter, Cupriavidus, Burkholderia, and Comamonas (Gunther *et al*., 2021). These microorganisms are commonly isolated from environments with prolonged exposure to herbicides, where they have developed adaptive metabolic mechanisms for survival and degradation of xenobiotic compounds (Singh et al., 2020; Li *et al*., 2023). The efficiency of microbial degradation is strongly influenced by environmental conditions such as pH, temperature, substrate concentration, and inoculum size, which directly affect microbial growth and enzymatic activity (Wang *et al*., 2024; Patel *et al*., 2024).

In Nigeria, particularly in Kura Local Government Area of Kano State, irrigation agriculture is widely practiced, and the continuous application of herbicides has led to significant environmental contamination. The soils in this region have been exposed to 2,4-D for several decades, creating a selective environment that favors the proliferation of indigenous microorganisms capable of degrading this compound (Shobana, 2017). Such environments serve as important reservoirs for isolating potent biodegrading bacteria with potential applications in environmental remediation (Adeyemi *et al*., 2023). Previous studies conducted in this region successfully isolated and characterized two distinct bacterial strains with high biodegradation potential. One study identified Cupriavidus campinensis as an efficient degrader, achieving up to 94.69% degradation under optimal conditions (Jahun *et al*., 2023). Another study reported Achromobacter xylosoxidans as a highly effective degrader, capable of degrading up to 95.38% of 2,4-D within a shorter incubation period (Jahun *et al*., 2024). Both isolates demonstrated the ability to utilize 2,4-D as a sole source of carbon and energy and exhibited optimal performance under similar environmental conditions, including neutral pH and moderate temperatures.

However, despite these promising findings, there remains a significant knowledge gap regarding the comparative performance of these isolates under identical experimental conditions. Comparative evaluation is essential for identifying the most efficient strain, understanding differences in degradation kinetics, and assessing their adaptability to environmental variations. Furthermore, comparative studies provide insights into the potential development of microbial consortia, which have been shown to enhance biodegradation efficiency through synergistic interactions (Pileggi *et al*., 2020; Lisiecka *et al*., 2024).

Therefore, this study aims to comparatively evaluate the biodegradation potential of Cupriavidus campinensis and Achromobacter xylosoxidans isolated from rice cultivated soils in Kura, Kano State, Nigeria. Specifically, the study assesses their degradation efficiencies, responses to environmental parameters, and suitability for application in sustainable bioremediation of 2,4-D-contaminated environments. The findings are expected to contribute to the advancement of microbial bioremediation

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strategies and support the development of environmentally sustainable approaches for managing herbicide pollution.

Materials and methods

Sample collection

Soil samples were collected from Kura, Bugau and Danhassan all in Kura local government area of Kano State and transported to Microbiology laboratory Bayero University Kano. All experiments were carried out in triplicates, experiments involving microorganisms were done in a class II safety cabinet (FOA, 2020). Glassware was washed with 2mM HNO_3 rinsed with deionized water. The brands of the High-Performance Liquid Chromatography, Polymerase Chain Reaction equipment, gel scanner, Deoxyribonucleic acid (DNA) isolation kit, Spectrum analyzer and electron microscope were utilized in this study (ISO, 2022). The reagents utilized for the study include polymerase, Agarose, 2, 4-D, nutrient agar, LB broth, and methylated spirit, 4NaCL, 1.74K_2HPO_4, 0.68$KHPO_4$, 0.1$MgSO_47H_2O$, 0.02CaCL.2H_2O, 0.03FeSO4.7H_2O, and 1.0NH_4NO_3. All the chemicals on the list are pure. The rice-growing area in Kura L.G.A. is located at (11◦46′17″N latitude and 8◦25′49″E longitude) Kano State. Provided the soils for the 2,4-D-resistance bacterial isolation and screening. The land has a known history of 2,4-D used over decades.

Methods

Isolation of bacteria that degrade 2,4-D

In a (250 mL) Erlenmeyer flask containing 50 mL of mineral salt medium, 5 g of soil was added as an inoculant. Before adding sterilized 2,4-D, the medium was autoclaved at 121 ◦C for 15 minutes. To prevent the production of precipitates following autoclaving, iron sulfate was added to the medium (Almeida *et al*., 2025). The medium was supplemented with 0.72 gL^{-1} 2,4-Das it served as the only source of energy and carbon. The flasks were shaken at 120 rpm in a rotary shaker for 96 hours at 30 ◦C. Afterward, 5mL of the culture media demonstrating 2,4-D breakdown was transferred to a 50mL flask containing new MSM enriched with 0.72gL^{-1} 2,4-D and then kept incubating for 96 hours. Repeated dilutions of the last culture were distributed on MSM plates containing 0.72 gL^{-1} 2,4-D after additional rounds of enrichment were completed. 4 days of inoculation at 30 ◦C later, colonies with various morphologies were chosen for additional evaluation of their degrading potential (Thakur *et al*., 2025).

Morphological and molecular identification

Strain B3-BUK-CH was identified and characterized based on the examination of its 16S rRNA gene sequence and biochemically. According to the Systematic manual of Bacteriology (Weerakkody & Witharana, 2024), biochemical and morphological analysis were carried out. Samples were set up for scanning electron microscopy. Genomic DNA was extracted using bacterial DNA Kit. The 16SrRNA genes were amplified using the universal primers F:5-'TGGAGAGTTTGATCCTGGCT CAG-3′ and R:5′-TACCGCGGCTGCTGGCAC-3′ At the department of Biochemistry Bayero University Kano (BUK), Kano's Instrumental Laboratory, microbial DNA extraction was carried out. An earlier approach by Mekonnen *et al*., (2024) was used to isolate the DNA. To discover the nearby strains with legitimate published prokaryotic names, the sequence was identified by blasting in the NCBI database (Pariseau *et al*., 2024). By using CLUSTALW version 2.0, several 16S rRNA genome from GenBank were assembled. MEGA6.0 software was used to create a phylogenetic tree (Bogožalec Košir *et al*., 2023). On the tree, there was a bootstrap analysis using 1000 replicates.

Characterization of the bacterial 2,4-D-degradation

The bacteria isolate B3-BUK-BCH was grown on Luria-Broth (LB) medium that had 0.72 gL^{-1} 2,4-D added to it. The cells were then washed with sterile MSM after being centrifuged and harvested

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at 6000 g for 5 min. At 120 rpm, flasks were put in a rotary shaker. The same procedures were employed to test un-inoculated medium as controls. At 76 hours, culture samples were taken out to evaluate cell growth at a 600nm optical density. The effects of initial 2,4-D concentration on growth and 2,4-D degradation were studied at various concentrations (0.36 gL^{-1}, 0.72 gL^{-1}, and 1.44 gL^{-1}), culture temperature (25–45 ◦C at 5 ◦C intervals), inoculums size (100–500 gL^{-1} at 100 gL^{-1} intervals), medium pH (5.5–8.5 with 0.5 interval), and incubation time (interval from 24 to 120h). Triplicates of each experiment were carried out. Using a spectrophotometer to gauge the sample's optical density at 600 nm, cell proliferation was quantified (Chakma *et al*., 2025). The amount of 2,4-D that was still present in the culture media was measured using HPLC to determine the degradation rate. The percentage of degradation was calculated using the 2,4-D standard curve. Cultures were preprocessed with the same techniques as used by Jahun *et el*., (2024).

Preparing samples for 2,4-D HPLC analysis

The procedure outlined in a study authored by Ref. (Geda *et al*., 2025), was followed when cleaning the samples for HPLC analysis. As described below. Dimethyl amine salt, or 2,4-D amine-salt solution, was bought from Samdaghe Nigewria Ltd. in Kano. Sigma Aldrich (Seelze, Germany) provided analytical-quality dichloromethane, HPLC grade acetone, methanol, acetonitrile, hexane, sodium sulfate, methanol, ethylacetate, hydrochloric acid, and chloroform. Schleicher and Schuell (Germany) provided the medium porosity Whatman filter paper number 1. Unless otherwise indicated, every other constituent was of analytical grade. With the G1311B quaternary pump, G1329B auto-liquid sampler, G1315C diode array detector, and G1316A thermal column compartment (TCC), the Agilent 1260 Infinity (USA) HPLC was utilized. Acetonitrile: water (75:25, 0.2 % formic acid) was the mobile phase, and it was used in an isocratic manner for 8 minutes. The auto sampler was used to make the injection, and in standard mode, the injection volume varies from 5 to 10 µL. The components were separated and identified using an Agilent-manufactured reversed phase C-18 column (UAS) with an internal diameter of 4 mm, a length of 250 mm, and a particle size of 5 µm. For 2,4-D determination, the DAD wavelength was set at 230 and 280 nm. Percentage degradation was calculated using the formular

$$\textit{Percentage degradation}\ (\%) = \frac{\textit{Initial Concentration - Final Conc.}}{\text{Initial Conc}}\ X\ 100\%$$

Statistical analysis

Tables and graphs were utilized to illustrate the findings. Bar charts, graphs, and one-way ANOVA with replications were used to show the characteristics of bacterial isolates at various time points (p 0.05) (Kim *et al*., 2024). Genstat was the statistical software program used.

Results and Discussion

Physical and chemical characteristic of the soil

The physical and chemical characteristics of rice cultivated soil obtained from Kura farm was presented in Table 1. Due to the fact that they can be used to draw conclusions about the results of these experiments, soil physicochemical parameters are crucial for biodegradation studies. The activity of the microorganisms that cause the mineralization of the pesticides is primarily dependent on these environmental factors (Usman *et al*., 2023). Additionally, some anomalies in the soil that may be noticed during analysis can be explained by these properties of the soil. The farm had acidic soil, as evidenced by the pH of the soil, which were 4.04. It has been discovered that some pesticides biodegrade slowly at pH values higher than 6 and optimally at pH values lower than 5 (Fatima *et al*., 2023). The study's results, which showed that 2,4-D degradation was slow at an acidic pH and reached its maximum at pH 7.5, conflict with this. The compound being broken down and the possible organism that breaks it down determine how the pH affects things, though. The degradation of pesticides is

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significantly influenced by soil temperatures. It has been observed that most pesticide degradation tends to increase within the range of 10 and 45 ◦C in temperature (Usman *et al*., 2023).

Since 2,4-D degradation was most effective at 40 ◦C, the results of study by Ref. (Aisha *et al*., 2023), were completely consistent with the results of this investigation where effective degradation was also achieved at the temperature of 40 ◦C at 96 h of incubation. The physicochemical characteristics of soil, including temperature, humidity, and moisture content, have an impact on how quickly herbicides break down in the soil (Jahun *et al*., 2022). The moisture content of the soil is very important for the degradation process (Huang *et al*., 2019). Water controls the pesticides' availability to microorganisms and serves as their solvent. In contrast to wet soil, dry soil typically has slower biodegradation. Anaerobic degradation has been observed in water-logged soil, as opposed to aerobic degradation because of the restriction of oxygen entry into the soil. However, depending on the pesticide in question, too much moisture content may speed up or slow down the utilization. On the other hand, prolonged pesticide use may negatively impact certain physical and chemical components of the soil. As per report by (Huang *et al*., 20219), the use of certain pesticides can result in modifications to the nitrogen (N2) fixing organisms, including Rhizobium, Azotobacter, and Azospirillum. Additionally, cellulolytic, and phosphate-solubilizing microbes may be impacted (Jahun *et al*., 2022).

Table 1. Physicochemical parameters of the soil sample from Kura rice cultivated soil

Variables	Measurements
%N	0.34 ± 0.03
P mg kg	1 23.79 ± 0.02
K mg kg	1 2.41 ± 0.01
Ca mg kg	1 0.55 ± 0.02
Mg mg kg	1 20.76 ± 0.04
pH	4.04 ± 0.37
Temp (ºC)	23.67 ± 0.88
% Moisture	22.35 ± 2.75
%TOC	22.35 ± 2.75
%WHC	76.02 ± 0.34
Texture	Sandy Clay

Water Holding Capacity (WHC), Total Organic Carbon (TOC). Data shows means ± SD, significance difference is at $p < 0.05$.

Isolation and screening of bacterial isolates

According to the study using 2,4-D as the only source of energy, six bacterial isolates coded (D1, D2, and D3) and (B1, B2 and B3) respectively in all were able to grow during the screening process when 2,4-D was present, while those with highest resistivity were chosen through the means of tolerant test by increasing the normal concentration of 2,4-D used from 0.72 gL^{-1} to 1.44 gL^{-1} to find the most withstanding strains based on its ability to tolerate high concentration of the substrate as the only carbon and energy source. Out of the six isolates two bacterial strains D2 and B3 shows higher optical density measurement of (0.272nm) and (0.247nm) respectively and therefore, were chosen for further identification and characterization studies. During inoculation at 30 ◦C for 3 days, Sequence analysis revealed that these strains' genomes had a group I tfdA gene's conserved sequence (Bernat *et al*., 2018). Among the 2,4-D-utilizing isolates, D2-BUK-BCH and B3-BUK-BCH strains demonstrated increased resistance and is the most effective at 2,4-D degradation.

Table 2. Tolerance test.

S/N	Isolate	Optical density (nM) (OD600rpm)
1.	D1	0.208 ± 0.03

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2.	D2	0.272 ± 0.01*
3.	D3	0.206 ± 0.02
4.	B1	0.205 ± 0.03
5.	B2	0.247 ± 0.04*
6.	B3	0.239 ± 0.01

Keys: D = Danhassan sampling station, B= Bugau, * indicate isolate with high optical density.

Identification of strains D2-BUK-BCH and B3-BUK-BCH

According to the results of biochemical tests and the morphology of the bacterial cell shown in Fig. 1 and 2, D2-BUK-BCH is a motile straight rod-shaped Gram-negative bacterium with oxidase and catalase activity and a negative indole and methyl red reactions while B3-BUK-BCH is a motile, rod-shaped Gram-negative bacterium with activity for oxidase and catalase tests for the use of glucose, citrate, and indole on the isolate yielded negative results. The strains D2-BUK-BCH and B3-BUK-BCH 16S rRNA respectively showed that they clustered with other bacteria in GenBank. As Achromobacter xylosoxidans and *Cupriavidus campinensis* with a 100% similarity index in their 16S rRNA gene sequences (Fig. 1 and 2 respectively). *C. campinensis* was discovered in Jordan Valley soil that had been treated to herbicides (Thakkar, 2021). Numerous varieties of 2,4-D-utilizing strains were discovered from 2, 4-D-contaminated areas in recent studies. *Alcaligenes, Achromobacter, Corynebacterium, Flavobacterium, Arthrobacter, Streptomyces,* and *Pseudomonas* are only a few of the genera of microbes whose ability to metabolize 2, 4-D has been described (Kaur *et al*., 2019; Carboneras *et al*., 2017). Recent investigations characterized these strains. In comparison to *Sphingomonas agrestis* strain 58-1, *Cupriavidus campinensis* has a greater degradation potential (Islam *et al*., 2018). Three isolates: *Sphingomonas paucimobilis* DS-3, *Pseudomonas* sp. DS-2, and *Burkholderia cepacia* DS-1 (Zharikova *et al*., 2018). A study by Muhammed *et al*., (2022) found that the medium supplemented with 2, 4-D, *Cupriavidus campinensis* as used in this study had a 2, 4-D degrading capacity comparable to that of *C. pampae* CPDB6 (Haruna *et al*., 2023) and *Halomonadaceae* spI-18. According to Amjad (2003), *Cupriavidus campinensis* had more potential of mineralizing 2, 4-D than *Pseudomonas putida*.

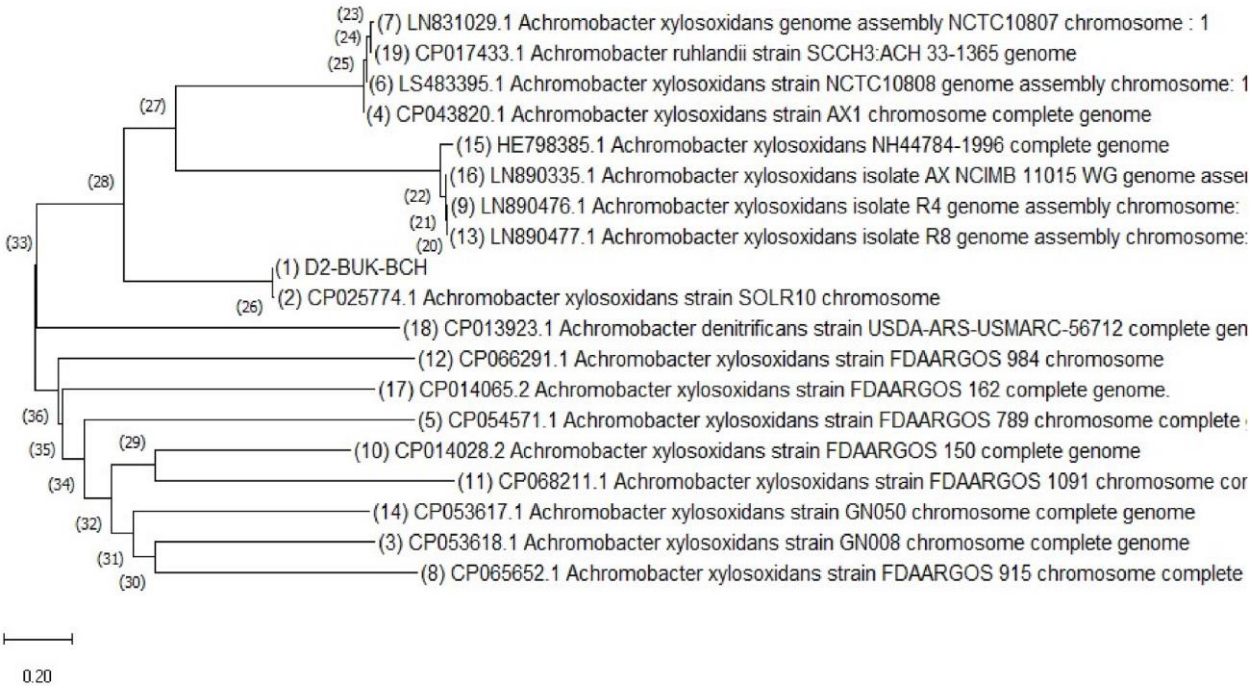

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Fig. 1 D2-BUK-BCH phylogenetic analysis displaying the correspondence sequences outcome with those deposited in the NCBI Genbank.

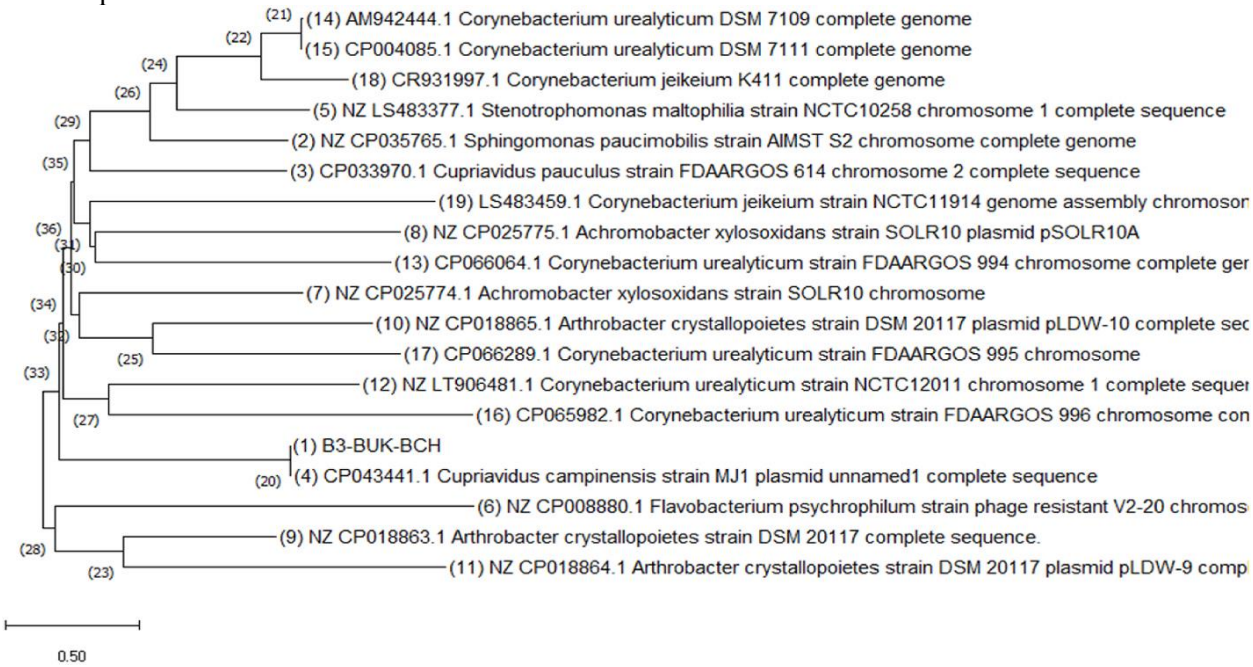

Fig. 2 B3-BUK-BCH phylogenetic analysis displaying the correspondence sequences outcome with those deposited in the NCBI Genbank.

Characterizations

Effect of substrate concentration on the growth of 2,4-D resistance bacteria

The comparative response of *Achromobacter xylosoxidans* and *Cupriavidus campinensis* to varying 2,4-D concentrations (Fig. 3) revealed a statistically significant variation ($p \leq 0.05$) in their growth and degradation efficiency across concentrations. At 0.35 g L^{-1}, both isolates showed comparable growth of (0.208 ± 0.03 and 0.205 ± 0.03 nM) respectively, indicating similar baseline tolerance. However, at 0.72 g L^{-1}, *A. xylosoxidans* exhibited significantly higher growth and degradation compared to *C. campinensis* (0.272 ± 0.01 and 0.247 ± 0.04 nM) respectively ($p \leq 0.05$), suggesting a more efficient substrate utilization mechanism. While at higher concentration (1.44 g L^{-1}), both isolates showed reduced activity with (0.206 ± 0.02 and 0.206 ± 0.02 nM) respectively, indicating substrate inhibition. This trend is consistent with previous studies reporting that high concentrations of 2,4-D exert toxic effects on microbial cells, thereby reducing degradation efficiency (Nascimentor *et al*., 2025).The optimal concentration (0.72 g L^{-1}) observed for both isolates aligns with earlier reports where *Cupriavidus* species showed maximum degradation at moderate substrate levels before inhibition at higher concentrations (Dhakal *et al*., 2025). Comparatively, the superior performance of *A. xylosoxidans* at intermediate concentrations suggests enhanced metabolic flexibility, possibly due to more efficient enzyme systems involved in phenoxy herbicide degradation.

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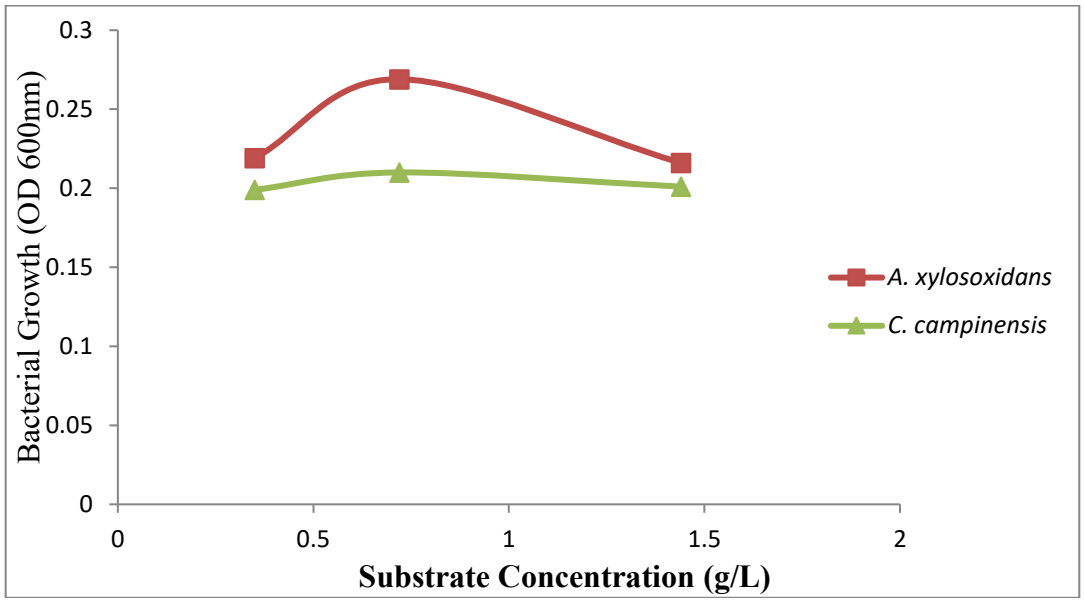

Fig. 3. Effect of the substrate concentration on the growth of 2,4-D degrading bacteria

Effect of pH on the growth of 2, 4-D resistance bacteria

The effect of pH on the growth of bacterial isolates *Achromobacter xylosoxidans* and *Cupriavidus campinensis* was evaluated. Fig. 4 shows that both the two isolates could resist 2, 4-D in a wide range of pH (5.5–8.0) with maximum growth at pH 7.5 and 7.0 respectively after which the growth patterns in both declines with increasing pH and therefore pH 7.5 and 7.0 were selected as the best pH for the growth and degradation activity. This slight variation indicates differences in enzyme stability and catalytic efficiency between the isolates. The preference for neutral pH is consistent with previous studies, which reported optimal 2,4-D degradation by Cupriavidus species at pH 7.0–7.5 (Stevenson *et al*., 2023). The soils from the farm had an acidic pH of 6.1, making it unsuitable for the isolate used in this study (Singh *et al*., 2020). The isolates exhibit greater activity in the pH range of 7–8, peaking at 7.5 and 7.0 respectively, making pH another important parameter to consider when remediation. Some chemical insecticides have been shown to degrade slowly at pH levels above 6 and remain stable at pH levels below 5 (Tan *et al*., 2016). However, the pH effect depends on the specific molecule being broken down and any potential organisms involved. This conclusion was in direct opposition to the findings of this investigation, which showed that the *A. xylosoxidans* and *C. campinensis* performed better at a pH of 7–8 and at its peak at 7.5 and 7 respectively. The decline in degradation at acidic and alkaline pH values suggests that enzymatic activity is inhibited outside optimal conditions. Notably, *A. xylosoxidans* maintained relatively higher activity at slightly alkaline pH, indicating broader pH tolerance and potential environmental adaptability (Sachu *et al*., 2023). Regrettably, *Pseudomonas cepacia's* breakdown 2, 4-D beyond the pH of 7 was sluggish and unchecked at low pH (pH, 3.3) or basic pH (pH, 8.1). The outcomes that followed coincide with our findings that CY-1 consumption of 2,4-D were more at an intermediate pH of 7.5, the result goes hand in hand with what is obtained in this study where *A. xylosoxidans* and *Cupriavidus campinensis* degraded up to 95.38% and 94.69% respectively of the 2, 4-D. Except for neutral conditions, where CY-1 displayed more activity, findings by Rose *et al*., (2018) were identical to our findings.

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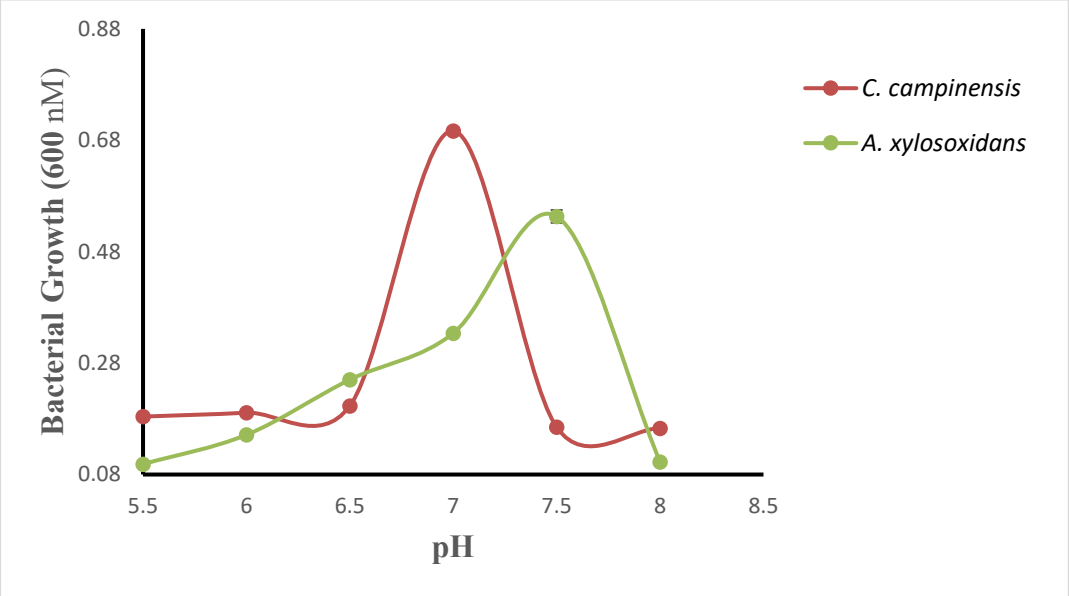

Fig. 4. Effect of pH on the growth of 2, 4-d resistance bacteria.

Effect of temperature on the growth of 2, 4-D resistance bacteria

The effect of temperature on the growth of 2, 4-D resistance bacterial strains *A. xylosoxidans* and *C. campinensis* was exhibited over the temperature ranges 30–45 ◦C. Growth was maximum at the temperature of 40 ◦C by both isolates confirming their mesophilic nature after that there was gradual decrease in the growth with increased temperature (Fig. 5). However, A. xylosoxidans consistently exhibited slightly higher degradation efficiency compared to *C. campinensis* at all temperatures tested. This suggests that *A. xylosoxidans* may possess more thermotolerant enzymes capable of maintaining catalytic activity at elevated temperatures. After 96 h of incubation up to 95.38 and 94.69% of the 2, 4-d was degraded respectively. The decline in activity at temperatures above the optimum is likely due to enzyme denaturation and reduced cellular stability. According to study by Noor, (2023), *Cupriavidus* had 2, 4-D degradation potential between 10 and 40 ◦C, with 30 ºC being the ideal temperature, this result was not in line with the finding in this study which might be due to differences in the environmental conditions, soil type as well as the physiology of the microorganism. But at 10 ºC, *Cupriavidus* only degraded somewhat (almost 80%). as a result, temperature has a big impact on how quickly compounds break down. according to research by Olusegun *et al*., (2018), chemical insecticides degrade more rapidly as temperature rises between 10 and 45 ˚C. herbicide breakdown rates in soil are influenced by physicochemical factors as humidity, temperature, and moisture content (Wang *et al*., 2023).

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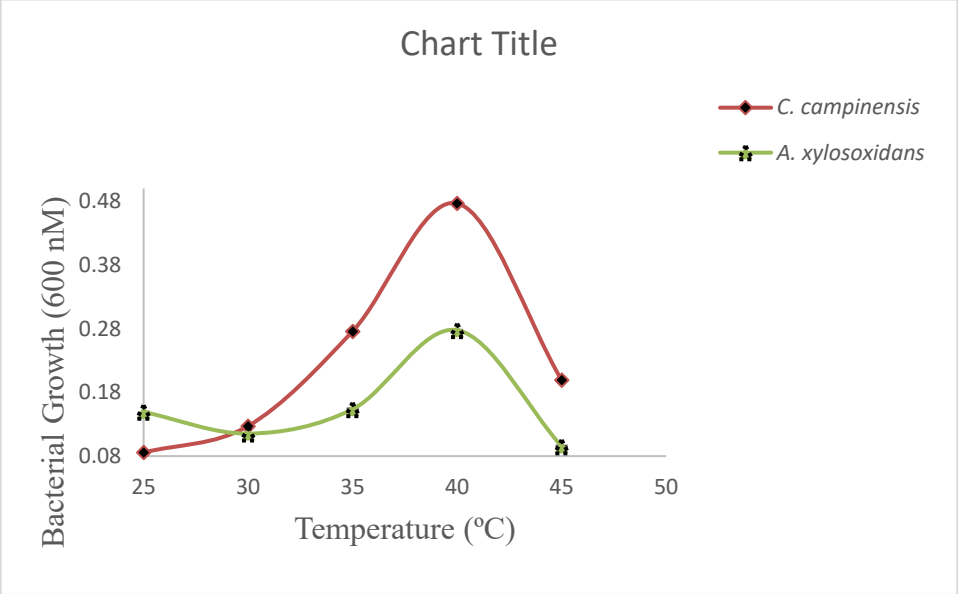

Fig. 5. Effect of temperature on the growth of 2,4-D resistance bacteria.

Effect of Incubation Time on the growth of 2, 4-D resistance bacteria

The impact of incubation period was also studied between the time intervals of 24 - 120h, and it was shown that the maximum growth was reached at 96 h, following which the growth began to diminish and therefore, 96 h was selected as the best incubation time for the 2, 4-D degradation by *Cupriavidus campinensis* (see Fig. 6). Comparatively, *Achromobacter xylosoxidans* demonstrated faster degradation kinetics, achieving near-complete degradation earlier than *Cupriavidus campinensis*. This suggests a shorter lag phase and faster enzyme induction in *A. xylosoxidans*. The plateau observed after 96 hours may be attributed to substrate depletion or accumulation of intermediate metabolites that inhibit further microbial activity. Studies have shown that degradation of 2,4-D typically follows a time-dependent pattern, characterized by an initial lag phase, an exponential degradation phase, and a stationary phase. During the lag phase, microorganisms adapt to the toxic environment and induce the expression of degradative enzymes such as those encoded by tfd genes. As incubation progresses, the exponential phase is marked by rapid degradation due to increased microbial biomass and enzymatic activity. Recent findings indicate that moderate incubation periods (4–12 days) are optimal for efficient degradation under laboratory conditions. For example, Rani *et al*., (2023) reported that approximately 81–90% degradation of 2,4-D occurred within 12 days, while increasing inoculum concentration reduced the required time to about 4–9 days for near-complete degradation, this result was similar with the findings in this study where both the two isolates *A. xylosoxidans* and *C. campinensis* achieved nearly 100% degradation after 4 days of incubation. This demonstrates that incubation time is closely linked with other factors such as inoculum size and microbial synergy. Similarly, studies on indigenous bacterial strains such as Cupriavidus campinensis have shown that up to 94.69% degradation can occur within 6 days of incubation, indicating that efficient strains can significantly shorten the required incubation period (Jahun *et al*., 2023). This highlights the importance of selecting high-performance bacterial isolates when optimizing incubation time.

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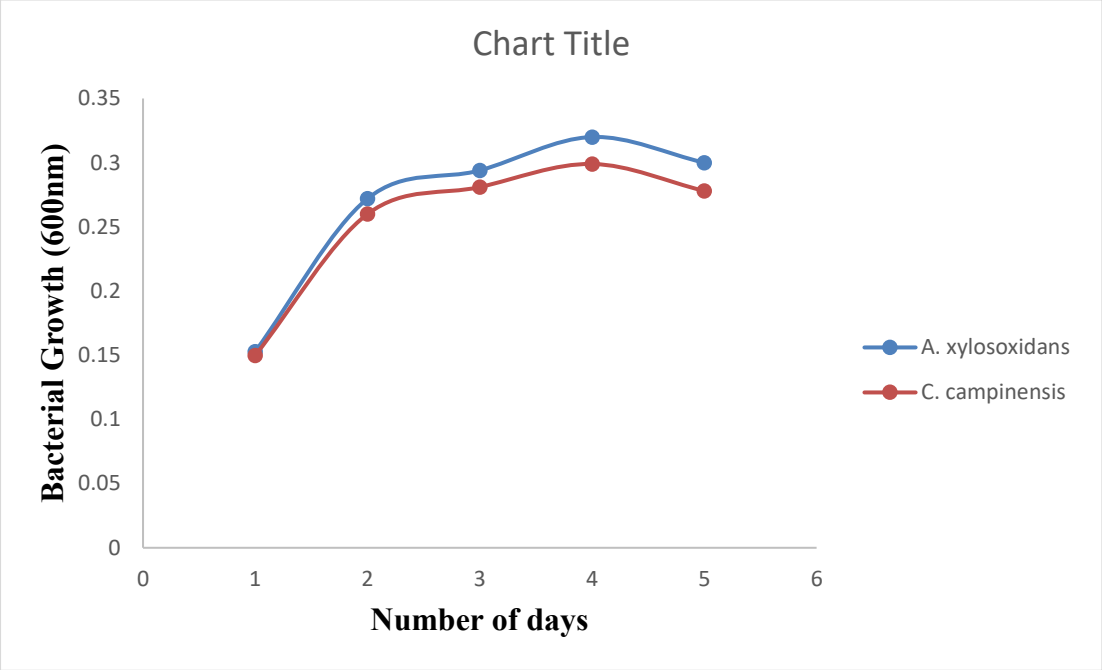

Fig. 6. Effect of incubation time on the growth of 2, 4-d resistance bacteria.

Effect of inoculum size on the growth of 2, 4-D resistance bacteria

The inoculums size was also another important parameter for the growth of 2, 4-D resistant bacteria. The growth pattern of the 2, 4-D resistant bacterial culture was monitored at different inoculums concentrations ranging from 100 to 500 μgL^{-1}. Over the period of time the turbidity of the medium for the two isolates *A. xylososxidans* and *C. campinensis.* The isolate grew more at 400 μgL^{-1} and 200 μgL^{-1} respectively than they did at rest of the inoculum concentrations Fig. 7. This variation reflects differences in growth dynamics and substrate utilization strategies. While higher inoculum size increases biomass and enzymatic activity, excessively high concentrations may lead to competition for nutrients and oxygen limitation. Optimization of inoculum concentration is a critical factor influencing the efficiency of microbial degradation of 2,4-dichlorophenoxyacetic acid in contaminated environments. The inoculum size determines the initial microbial population available for substrate utilization and directly affects degradation kinetics, lag phase, and overall biodegradation efficiency. Recent studies have demonstrated that increasing inoculum concentration significantly enhances the degradation rate of 2,4-D. For instance, a study by Rani *and Kumar*, (2024) showed that at a 2% inoculum, bacterial isolates achieved 81–90% degradation within 12 days, whereas increasing the inoculum to 10% reduced the lag phase and achieved ~80% degradation within 5 days. This indicates that higher inoculum density accelerates microbial adaptation and enzymatic activity, thereby shortening the time required for effective biodegradation, the result agree with the result in this study where *C. campinensis* degrade up to 94.68% of 2,4-D after 4 days of incubation and slightly differ with what is obtained by *A. xylosoxidans*. Furthermore, optimization of inoculum concentration becomes more effective when combined with microbial consortia rather than single strains. Mixed cultures at 10% inoculum concentration demonstrated 85–90% degradation within 4 days and nearly complete degradation (98–100%) within 9 days, outperforming axenic cultures (Chen *et al*., 2024). This improved performance is attributed to synergistic interactions among different bacterial species such as *Arthrobacter*, *Sphingomonas*, and *Pseudomonas*, which enhance metabolic diversity and breakdown pathways. In addition to degradation rate, inoculum concentration also influences substrate availability and competition. At low inoculum sizes, microbial populations may be insufficient to utilize the

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available 2,4-D effectively, resulting in prolonged lag phases and incomplete degradation. Conversely, excessively high inoculum concentrations may lead to nutrient depletion, oxygen limitation, and metabolic competition, which can reduce overall efficiency (Jahun *et al*., 2023). Therefore, an optimal inoculum range is necessary to balance microbial activity and environmental conditions.

2,4-D degradation (%)

The percentage degradation was calculated using the formula and it was found that *C. campinensis* degrade up to 94.69% while *A. xylosoxidans* degrade up to 95.38% of the substrate under optimal conditions respectively as calculated below.

Percentage Degradation = $\frac{\text{Initial - Final Conc.}}{\text{Initial Conc.}} X\ 100\%$

Percentage Degradation of *C. campinensis* = $\frac{0.72 - 0.0382}{0.72} X\ 100\%$

Percentage degradation *C. campinensis* = 0.688 / 0.72 = 94.69%

Percentage degradation of *A. xylosoxidans* = $\frac{0.72 - 0.0332}{0.72}$ x 100 = 95.38%

Conclusion

This comparative study on the biodegradation of 2,4-dichlorophenoxyacetic acid (2,4-D) by *Achromobacter xylosoxidans* and *Cupriavidus campinensis* demonstrated that both bacterial species possess significant potential for the bioremediation of this herbicide-contaminated environment, although their degradation efficiencies varied under the tested conditions.

The results indicated that *Cupriavidus campinensis* generally exhibited a higher rate of 2,4-D degradation compared to *Achromobacter xylosoxidans*, suggesting a stronger metabolic capability or greater enzymatic efficiency in utilizing 2,4-D as a carbon source. However, *Achromobacter xylosoxidans* also showed appreciable degradation activity, confirming its role as a competent degrader and its potential usefulness in mixed or consortium-based bioremediation systems.

Overall, the study confirms that both bacterial isolates can contribute effectively to the reduction of 2,4-D contamination in the environment, with performance influenced by factors such as incubation time, pH, and substrate concentration. The findings support the application of indigenous microbial strains as eco-friendly and sustainable alternatives to conventional chemical remediation techniques.

Further research is recommended to optimize environmental conditions, investigate enzyme pathways involved in degradation, and explore the synergistic potential of both organisms in a co-culture system for enhanced biodegradation efficiency.

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