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Achromobacter sp. Strain BUK_BCH_TQ1: A Potential Paraquat-Degrading Bacterium Isolated from Pesticide Contaminated Agricultural Soil

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ABSTRACT

Paraquat (1, 1′-dimethyl-4, 4′-bipyridinium dichloride) is one of the most frequently used herbicide in agriculture. It is a cationic non-systematic, non-selective contact compound that instantaneously interferes with the photosynthetic processes of plants. It has an immediate effect, once the compound comes into contact with the plants' leaves, where the reaction occurs. However, the contamination of paraquat residue in soil can harm soil microbes, flora and fauna, farmer health and also soil ecology, which affects the soil fertility. The objective of this study was to isolate and characterize bacteria with the ability to break down and utilize paraquat as the primary carbon source. The isolation process involved the enrichment of mineral salt media (MSM) using serial dilution. The isolated bacterium underwent morphological, biochemical, and molecular identification following characterization. Results showed the isolate was identified as *Achromobacter sp.* with the accession number OQ372943 based on partial 16S rRNA gene sequence and phylogenetic analysis. The growth and degradation of paraquat by this isolate were optimum at a pH of 6.5, 276 mgL^{-1} of the substrate (paraquat), temperature of 35 °C, 200 µL of biomass size, and 48 h of incubation. The degradation efficiency of the isolate after 120 hours of incubation under optimal conditions was 91.01%. Hence, these results demonstrated a very high efficiency of paraquat degradation. Consequently, this isolate holds significant promise for paraquat degradation and could serve as a viable contender for remediating paraquat-contaminated environments.

1. INTRODUCTION

The tremendous increase in the world population causes higher demand of food and consequently it has led to sustainability of food production through improvement with advanced agricultural technology. This technology entails the use of herbicide and pesticides among others. However, while the technology efficiently enhances food production, but at the same time, the detrimental effect of some of the methods used is manifesting in both biotic and abiotic components of the ecosystem including humans (1). Increase in the production and application of agricultural and industrial chemicals have caused rapid increases in contaminated soils and waters (2, 3). Modern agriculture relies heavily on the use of herbicides for the control of weeds and ease out to maximize yield in crops. Herbicides are inorganic and biological agents used in weed control (4). Herbicide application has significantly curtailed labor demands and mitigated soil erosion by minimizing repetitive cultivation.

Paraquat (1,1′-dimethyl-4, 4′-bipyridinium dichloride) is an organic compound with the molecular formula $[(C_6H7N)_2]Cl_2$. It acts rapidly and indiscriminately, eradicating green plant tissue upon contact by disrupting plant photosynthesis. Its redox activity renders it hazardous to humans and animals, generating superoxide anions and links

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to Parkinson's disease (5). In the United States, paraquat is available primarily as a solution in various strengths. It is classified as "restricted use" which means that it can be used by licensed applicators only (6). There is an ongoing international campaign for a global ban, but the cheap and therefore popular paraquat continues to be unrestricted in most developing countries like Nigeria (7).In this context, the solution lies in employing inexpensive, efficient, and environmentally friendly processes, with microbial bioremediation emerging as a viable answer to these issues.

Currently, one of the best emerging technologies employed to clean up environment and with less side effect is through the use of microorganisms, a technique popularly referred to as bioremediation (8). Microorganisms widely dispersed in nature possess the capability to utilize herbicides as exclusive sources of carbon and energy, thereby converting these herbicides into harmless byproducts like water and carbon dioxide (9).. Research has confirmed that specific bacterial and fungal species possess the ability to degrade paraquat in soil and slurry environments. Several bacterial strains, such as *Bacillus cereus*, *Aerobacteraerogenes*, *Pseudomonas fluorescens*, *Agrobacterium tumefaciens*, and, have been identified due to their capacity to break down paraquat (10). These bacteria can use the compound as a sole source of carbon or nitrogen for growth (11). Similarly, bacteria like, *Sporohalobacterorenetal* BCK-3, *Clostridium prazmowski* BCK- 2 and *Oscillospira sp*. BCK-1 have demonstrated effective degradation of paraquat (9). Remarkably, they achieved degradation rates of up to 79.35%, 80.26%, and 86.22%, respectively, within a 72 hours treatment period (9). The rate of biodegradation under controlled conditions by bacterial or fungal strain has been proven to be influenced by various environmental factors including; temperature, pH, nutrient availability, initial concentration of the substrate, and biomass dosage (12). Other factors such as the presence of electron donors and activated charcoal were also found to increase the degradation capability of paraquat by these organisms (13). Using microbial consortium capable of paraquat degradation was also found to increase degradation rate when compared with only one organism (10).

Looking at the detrimental effect of paraquat on the environment, humans and other animals, it became necessary to continue to search for microorganisms capable of degrading this compound with minimal harmful by-products. Furthermore, looking at the fact that environmental factors greatly played a role in the biodegradation of a particular pollutant, it then became necessary for the continual search of organisms in different environments. In addition, combination of microbial consortium has proven to be more effective for biodegradation of a particular compound. Therefore, continual isolation and characterization of various herbicide and pesticide degrading organisms is vital for effective removal of these substances. Hence, the present research aimed to isolate and molecularly identify bacteria capable of breaking down paraquat from agricultural land in Kura Local Government Area of Kano State, Nigeria. Additionally, the study sought to quantify the extent of paraquat degradation using High-Performance Liquid Chromatography. It is hoped that this study will provide an insight into the presence Paraquat degrading bacteria in this particular environment that can be utilized for bioremediation of this compound.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Study Site

The study was conducted within the geographic confines of Kura Local Government Area, situated at the coordinates of 11º46’17” N latitude and 8º25’49” E longitude in Kano State, Nigeria. Kano State spans across a total land area of 20,760 square kilometers, with 1,754,200 hectares recognized for their rich agricultural capabilities. Within this area, 86,500 hectares are specifically designated as rice cultivating land, while approximately 75,000 hectares serve as grazing grounds.

2.1.2 Chemicals and Reagents

All the chemicals and reagents employed in this investigation were of the utmost purity and adhered to the standards of analytical grade. The culture media were formulated following the procedures outlined by (14). All procedures involving microorganisms were conducted within a class II biosafety cabinet to ensure safety and proper containment.

2.2 Methods

2.2.1 Sample Collection

A soil sample was collected from rice field located at Kura local government area of Kano State. The rice field has a long history of using Gramaxone-based formulation whose active compound is paraquat. Soil sample (100g) was collected with a sterile scoop from a depth of 10cm from four different points in the same location. The soil sample collected from the site was carefully blended and placed inside a sterile polythene bag. Subsequently, it was promptly transported to the Microbiology laboratory of Bayero University, Kano, for the purpose of isolating and characterizing the microorganisms present within it.

2.2.2 Isolation of Paraquat Degrading Bacteria by Enrichment Method

The soil enrichment medium was manually prepared by a technique described by Gimba et al., (2022) (14) was employed in this study. All chemicals used were of a high grade purchased from Sigma Aldrich USA. A total of 150 ml of mineral salt medium (MSM) was poured into a 250 ml conical flask. The composition of MSM per liter included: 3 g of NH_4NO_3, 1.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.2 g of $MgSO_4.7H_2O$, 0.5 g of NaCl, 0.02 g of $FeSO_4$, 0.05 g of $CaCl_2$, with a pH adjusted to 7. Prior to the introduction of paraquat, the medium was autoclaved at 121ºC for 15 minutes. For the enrichment process, 10 grams of the soil sample were placed in a 250 ml conical flask, which was then supplemented with 150 ml of mineral salt medium. To serve as the exclusive carbon and energy source, 1% of paraquat herbicide was added. The culture was subsequently incubated at a temperature of 37°C using a Wise Cube Shaking incubator, set to 120 rpm, for duration of 7 days.

2.2.3 Inoculum Preparation

Following a 7-day incubation period, a 10 mL portion of the enriched culture was transferred to separate 250 mL conical flasks. These flasks contained 90 mL of freshly autoclaved MSM broth, enriched with 1% paraquat as detailed earlier. The mixtures were then subjected to agitation and incubated for an additional 7 days, as previously indicated.

Subsequently, each culture underwent serial dilution, ranging from 10^{-1} to 10^{-6}, utilizing sterile distilled water. Dilutions in the range of 10^{-5} to 10^{-6} were subsequently plated on MSM agar supplemented with 1% Paraquat (276 mg/L). These plated Petri dishes were then subjected to 48-hours incubation at 37 °C. After the requisite incubation period, the resulting colonies were subject to repeated sub-culturing on sterile MSM agar to ensure purification. Once purification was accomplished, the retrieved isolates were cultivated on nutrient agar slants, serving as stock cultures, and stored at a temperature of -20ºC (14).

2.2.4 Screening of Paraquat Degrading Bacteria

From the soil sample contaminated with paraquat, a total of six bacterial isolates, designated as TQ_1, TQ_2, TQ_3, TQ_4, TQ_5, and TQ_6, were successfully isolated. Tolerance of the bacterial isolates was tested using ¼, ½, 1, and 1.5% of the Paraquat which is equivalent to (69 mg/L, 138 mg/L, 276 mg/L, and 414 mg/L) respectively. All the six isolates displayed a degree of tolerance towards these varying concentrations, indicating a natural adaptation likely resulting from their isolation from a paraquat-contaminated sample. However, only one isolate, TQ1, exhibited complete tolerance to the paraquat concentration after five days of incubation. As a result, this isolate was selected for further investigation.

2.3 Identification of Isolated bacteria

2.3.1 Morphological Identification

A sample was applied to a slide devoid of grease, and subsequent heat fixation was conducted. This treated sample was then positioned on a staining rack and subjected to a 60-second immersion in crystal violet solution. Following this, the slide underwent a thorough water rinse to remove any excess solution and prevent dilution of the mordant. The sample was further subjected to a 30-second immersion in an iodine solution (serving as the mordant) and then rinsed with water. Decolorization was accomplished using alcohol, followed by a subsequent water rinse. Moving forward, the slides were inundated with safranin solution for a period of 60 seconds and then rinsed once more with water. After these steps, the slides were allowed to naturally dry in the air. The next stage involved the examination of the slides using an oil immersion microscope, utilizing an x100 objective lens. In this examination, Gram-positive bacteria exhibited hues ranging from blue to purple, while Gram-negative bacteria were characterized by shades spanning from pink to red (15).

2.3.2 Molecular Identification

2.3.2.1 Genomic DNA Extraction

Genomic DNA extraction was carried out from a pure bacterial culture that had been cultivated on Luria-Bertani medium at a temperature of 35°C. After cultivation, the culture was subjected to centrifugation at 5000 rpm for duration of 2 minutes using the Hettich Centrifuge Micro185 from Germany. The resulting pellets obtained from the centrifugation step were then homogenized in 100 µL of Livak grind buffer that had been warmed. The mixture was incubated at 65°C for a period of 30 minutes, using a DLAB Hem 1000 pro-USA heating block. Following the incubation, 14 µL of 8M K-acetate was introduced to the mixture to achieve a final concentration of 1 M. This was followed by 30-minute incubation on ice packs. Subsequently, the mixture underwent centrifugation for duration of 20 minutes at a temperature of 4°C. The resulting supernatant was carefully transferred to a new 1.5 cm^3 Eppendorf tube. To this supernatant, 200 µL of 100% ethanol was added, and the mixture was vortexed using an IR StarlabVortexer from Italy. After eliminating the supernatant, the remaining pellet was washed with 100 µL of ice-cold 70% ethanol. Following the drying of the pellet, it was resuspended in 100 µL of dH_2O and then incubated at a temperature of 65°C for 10 minutes (14).

2.3.2.2 DNA Amplification (Polymerase Chain Reaction)

Polymerase Chain Reaction (PCR) was used to amplify gene fragments targeting a highly conserved region of the bacterial 16s rRNA gene (16). The universal primers, 16s forward and 16s reverse, were acquired from Sigma Aldrich USA, with specific sequences as follows: 5'-TGGAGAGTTTGATCCTGGCTCAG -3' for the forward primer and 5'- TACCGCGGCTGCTGGCAC -3' for the reverse primer. For the PCR reaction, the reaction mixture had a total volume of 50 µl, consisting of 2.5 µl of (10µM) each primer , 10X buffer (5 µl), 4 µl of 2.5 Mm dNTPs, 3 µl of genomic DNA template (47ng/µl), 2 µl of 50 mM $MgCl_2$, and 5U/µl Taq DNA polymerase (0.2 µl). The PCR amplification process was initiated with a denaturation step at 95°C for 5 minutes, followed by 30 cycles, each comprising denaturation at 94°C for 1 minute, primer annealing at 55°C for 1 minute, primer extension at 72°C for 1 minute, and a final extension at 72°C lasting for 10 minutes.

2.4 Gel Electrophoresis

The DNA fragments that underwent amplification were separated using agarose gel electrophoresis. This was achieved by utilizing a 1% agarose gel and a 0.5X Tris/borate/EDTA buffer. Following the separation process, the resulting DNA products were treated with ethidium bromide for staining. Subsequently, the stained products were appropriately diluted using distilled water. Visual inspection of these products was performed, and their images were captured using a gel-documentation system (2).

2.5 Sequencing, Alignment and Phylogenetic Analysis

After the successful amplification of the DNA fragments, the accurately sized products underwent a purification process. Subsequently, bidirectional sequencing was carried out using an ABI automated sequencer. The 16S rRNA gene sequences obtained from the isolated strains were aligned and juxtaposed with sequences already archived in the GenBank database. This comparative analysis was executed using the Basic Local Alignment Search Tool (BLAST), which is accessible on the National Center for Biotechnology Information (NCBI) website at http://www.ncbi.nlm.nih.gov/BLAST/. The 16S rRNA gene sequence was then officially deposited in the NCBI with the GenBank accession number OQ372943. To align the partial 16S rRNA gene sequence of the isolate with relevant sequences from the GenBank database, we utilized the Clustal Omega tool for multiple sequence alignment. Following this alignment, we used the Neighbor-Joining method to infer the evolutionary history, with branch supports determined through a bootstrap test comprising 1000 replicates. To construct the phylogenetic tree, Molecular Evolutionary Genetics Analysis (MEGA) version 11.0 software for Windows was used (17).

2.6 Characterization of Paraquat-Degrading Bacteria

The growth of microorganisms is influenced by a range of environmental factors. To comprehensively investigate their

impact on the degradation of paraquat, a series of triplicate experimental runs were performed employing a one-factor-at-a-time (OFAT) approach (18). This study aimed to analyze the effects of variables such as pH, temperature, inoculum size, incubation period, and concentration of the substrate (paraquat) on achieving optimal paraquat degradation.

2.6.1 Effect of Incubation Time on Paraquat Degradation

The optimum incubation time was achieved by inoculating the isolate in 100mL of MSM amended with 1% paraquat for a period of 120 hours in an incubator shaker at 120rpm and readings were taken every 24h to determine the optical density at 600 nm.

2.6.2 Effect of pH on Paraquat Degradation

To examine the influence of pH on the degradation process, MSM supplemented with 1% paraquat was adjusted to varying initial pH levels within the range of 5.5 to 8.0. This pH manipulation was achieved using 1M HCl and 1M NaOH solutions. Subsequently, 100 µL of the bacterial suspension was introduced into separate 250 mL conical flasks, each containing 100 mL of sterilized MSM. These setups were then subjected to incubation at 37°C under shaking conditions (120 rpm). In parallel, a control setup without bacterial inoculation was maintained under identical conditions. At defined intervals (every 24 hours), samples of the bacterial culture were collected, and their absorbance was measured at 600 nm over a period of up to 120 hours.

2.6.3 Effect of Inoculum Size on Paraquat Degradation

To investigate the impact of inoculum size on the degradation of paraquat, 100 mL of MSM enriched with 1% paraquat was subjected to inoculation with varying quantities of the bacterial suspension: 50 µL, 100 µL, 200 µL, 300 µL, and 400 µL (1 mL = 1.45 * 10^6 cfu). The mixtures were subsequently placed under incubation at 37°C. At predefined intervals of time (every 24 hours), samples of the bacterial culture were retrieved, and their absorbance was gauged at 600 nm over a period of up to 120 hours.

2.6.4 Effect of Substrate Concentration on Paraquat Degradation

The investigation into the influence of substrate concentration on paraquat degradation involved utilizing 100 mL of MSM enriched with varying concentrations of paraquat, ranging from 69 mg/L to 414 mg/L. The culture flasks were subsequently inoculated with 100 µL of the bacterial suspension and subjected to incubation at 37°C while being agitated at 120 rpm. As a comparative measure, control setups were maintained without the substrate under identical incubation conditions. At defined intervals (every 24 hours), samples of the bacterial culture were collected over a period of up to 120 hours. Subsequently, the absorbance of these samples was assessed at 600 nm using a spectrophotometer.

2.6.5 Effect of Temperature on Paraquat Degradation

To study the effect of temperature on degradation, 100 mL of MSM was freshly prepared into 250 mL of conical flasks and supplemented with 1% of Paraquat. The isolated bacterial suspension of 100 µL (1 mL = 1.45 * 10^6 cfu) was added into various conical flasks and was then incubated at various temperatures ranging from 25-45 ˚C (25, 30, 35, 40, and 45) [23]. An aliquot was collected at regular intervals of time (24 h) for up to 120 hours and absorbance was measured using a spectrophotometer at 600nm.

2.7 High Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) was carried out utilizing an IZA HPLC system, specifically the LC-600A model produced in India. The methodology was aligned with the procedure outlined by Mohebi et al., (19), which involved employing a liquid-liquid extraction technique. The HPLC system encompassed components such as a pump, column oven, detector, data acquisition software, and supplementary accessories. For the liquid-liquid extraction, 2 mL of ethyl acetate was introduced to 1 mL of the MSM-bacterial sample, followed by vortexing for duration of 1 minute. After allowing the sample to settle, ensuring the separation of the aqueous and organic phases, the upper layer of the aqueous phase was carefully isolated using a pipette and transferred to a separate test tube. The organic solvent was subsequently evaporated through a rotary evaporator, and the resultant residues were reconstituted using 1 mL of HPLC-grade methanol. These reconstituted samples were stored at 4°C before undergoing HPLC analysis.

2.8 Percentage Degradation of Paraquat

The degradation of paraquat was studied through HPLC analysis. In order to determine the percentage (%) degradation by the isolated strains, samples together with control solutions were analyzed by High performance liquid chromatography (HPLC).

2.9 Data Analysis

All experimental procedures were performed in triplicate to ensure reliability. The deviations stemming from experimental variations are visually represented through error bars, which depict the standard deviation across three measurements. For data analysis, Microsoft Excel and GraphPadInStat version 3.05 were employed.

3. RESULTS AND DISCUSSIONS

3.1 Isolation and Screening

The result of tolerance test of the isolates is presented in table 1. A total of six bacterial isolates labeled TQ1, TQ2, TQ3, TQ4, TQ5, and TQ6 were screened for their tolerance to paraquat. Out of the six isolates, only one (TQ1) was found to grow in the highest concentration of the Paraquat employed (414 mg/L) and therefore was chosen for further study.

Table 1: Paraquat Tolerance Test of Bacterial Isolate Grown on MSM after 24 hours of Incubation at 37 ℃.

Isolate	***Paraquat concentration (mg/L)***			
	69	***138***	***276***	***414***
TQ_1	+	+	+	+
TQ_2	+	+	+	-
TQ_3	+	+	_	_
TQ_4	+	+	+	_
TQ_5	+	_	_	_
TQ_6	+	+	+	_

KEYS: + = indicate growth - = Indicate no growth

3.2 Identification of Paraquat-Degrading Bacteria

The result of morphological and biochemical identification of (TQ1) isolate is presented in table 2. Morphological identification of the bacterial isolate from revealed that isolate TQ1 was gram negative and rod shaped.

Biochemical tests revealed that TQ1 is positive for oxidase, catalase, starch utilization, urease, nitrate reduction and citrate tests. It was found out to be negative for indole, methyl red, and Voges-Proskauer. A finding by Huang et al., (2019) (10) reported that the majority of paraquat degrading bacteria isolated from soils were gram-negative rod and cocci-shaped. An earlier investigation conducted by Teerakun et al., (2020) (20) highlighted the prevalence of gram-negative bacteria in paraquat-contaminated soils. This predominance has been attributed to the specific characteristics of their membranes which contribute to accelerating the biodegradation process.

Table 2: Morphological and Biochemical Characteristics of the Isolate Used for Characterization and Paraquat Degradation Study.

Biochemical Test		Morphological
Gram Reaction	-	Rod shaped
Indole test	-	
Methyl red	_	
Oxidase test	+	
Catalase test	+	
Voges-Proskauer	_	
Nitrate reduction test	+	
Urea utilization test	+	
Citrate Utilization test	+	
Starch Utilization test	+	

Keys: + =Positive; - =Negative

Identification of the bacteria was further conducted based on the analysis of a partial segment of the 16S rRNA gene. The gel electrophoresis results for the partial 16S rRNA gene of the isolates revealed that the DNA sequences derived from the 16S rRNA analysis of isolate TQ1 exhibited a strong resemblance to the partial sequences of numerous Achromobacter species, with a similarity level up to 98.86%. Through phylogenetic analysis, the obtained sequences were associated with the identity of *Achromobacter* sp. strain P6, Figure 3. As a result, the bacterium was provisionally classified as *Achromobacter* sp. Strain BUK_BCH_TQ1, with the corresponding GeneBank accession number OQ372943. Different genera of bacteria capable of Paraquat biodegradation were identified in many other studies. These include genera such as *Sphingomicrobium marinum*, *Ferrovibrio xuzhouensis*, *Azospirillum lipoferum*, *Altererythrobacterinangensis*, *Xanthobacter.autotrophicus*, *Pseudomonas geniculata* and *Azospirillum amazonense* (21, 22). The diversity of Paraquat degrading bacteria may be attributed to the differences in the environmental conditions where these organisms were isolated as each organism may be well adapted and suited to a particular environmental condition.

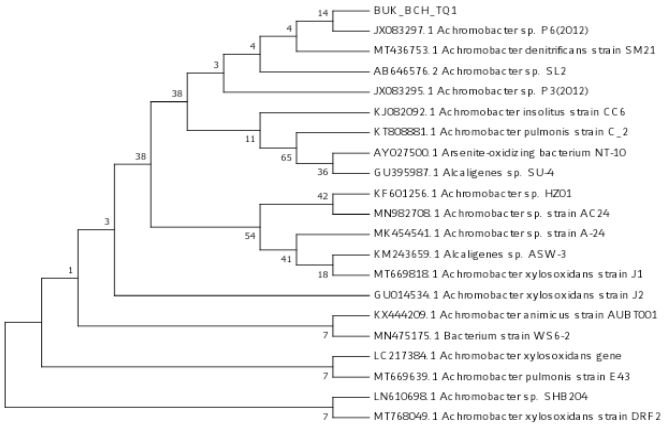

Fig. 1. Evolutionary relationship of BUK_BCH_TQ1 using neighbor –joining tree

3.3 Characterization of growth conditions for paraquat degrading bacteria

3.3.1 Effect of incubation time on paraquat degradation

The influence of various incubation times on the growth of Paraquat-degrading *Achromobacter sp* strain BUK_BCH_TQ1 in MSM was also studied and presented in figure 2. Notably, optimal bacterial growth was observed at the 48-hour mark following incubation at 37°C. A pronounced significant ($p<0.05$) decline in growth was evident when the incubation period surpassed 48 hours. Based on the outcome above, the optimum incubation time for the degradation of paraquat from this study was found to be at 48 hours (Figure 2). Research by (23), has reported the biodegradation of paraquat within 3-7 days of incubation at 30ºC. Similarly, other studies have reported complete biodegradation of paraquat within 96 hours of incubation (24). A study by (25) reported biodegradation of paraquat at 48 hours. Differences in the capacity of paraquat degradation between bacteria may have been related to inter and intra-specific variations, the molecular complexity of the herbicide, and the culture condition (10). Furthermore, environmental conditions where these organisms were isolated could play a big role in the differences of their biodegradation capabilities.

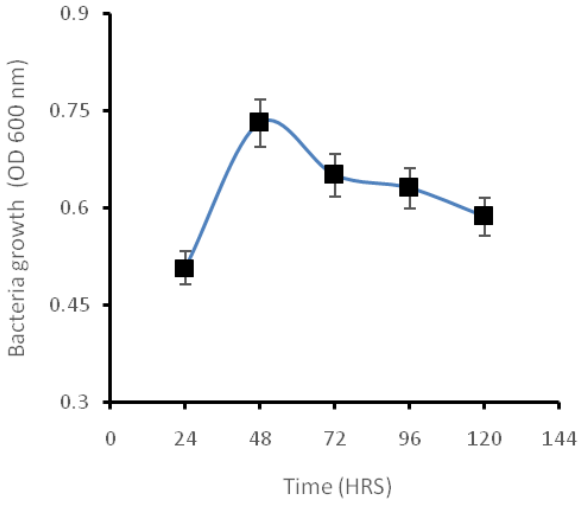

Fig. 2. Effect of Incubation Time on the Growth of Paraquat-degrading Bacteria Grown on MSM at 37 ºC after 120h of Incubation.

3.3.2 *Effect of initial pH on paraquat degradation*

Figure 3 depicts the influence of various pH on the growth of paraquat-degrading bacteria in the MSM media. Notably, the optimal growth of *Achromobacter* species strain BUK_BCH_TQ1 occurred at a pH of 6.5, after a 48-hour incubation period at 37°C. Beyond this pH threshold, there was a marked and statistically significant ($p<0.05$) reduction in growth, particularly when pH surpassed 7.0. The pH of the surrounding environment plays a crucial role in the microbial degradation of paraquat when it serves as the sole carbon and energy source. While many microorganisms thrive under neutral pH conditions, exceptions exist, including certain *Archaea* that can withstand extreme acidity or alkalinity. Adapting to pH stress often involves physiological changes in the cell membrane to aid in regulating intracellular pH. The current study investigates how pH impact bacterial growth and paraquat degradation across a range of pH values, spanning from 5.5 to 8. Among these values, an optimal pH of 6.5 was identified for *Achromobacter* species strain BUK_BCH_TQ1. Several other studies have also pointed to optimal growth occurring at pH 7 (26). Furthermore, research conducted by (27) found the optimal pH for *Pseudomonas* to be 6.5 during paraquat biodegradation in MSM. The slight variation in pH across the different genera of bacteria may be as a result of the differences in the environmental condition where the bacterium is isolated.

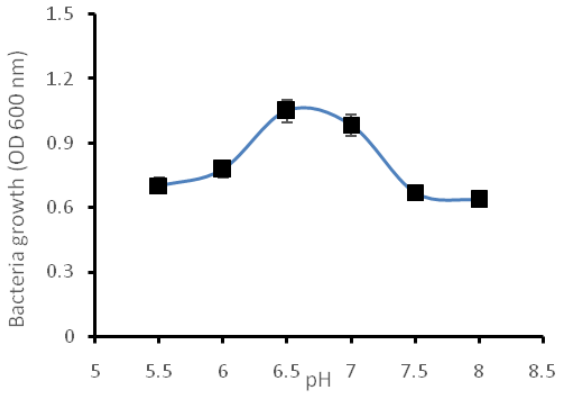

Fig. 3. Effect of initial pH on Paraquat-degrading *Achromobacter sp* growth in MSM Media Supplemented with Paraquat at incubated for 48 hours at 37 °C.

3.3.3 *Effect of Substrate Concentration on paraquat Degradation*

Figure 4 illustrates the influence of varying substrate concentrations on the growth of Paraquat-degrading *Achromobacter sp.* strain BUK_BCH_TQ1 in MSM media. There was significant rise in growth and degradation as the paraquat concentration increases, attaining optimal growth at a substrate concentration of 276 mg/L. This was maintained for up to 48-hours. Beyond this threshold, a marked and significant ($p<0.05$) reduction in growth was observed. A significant ($p<0.05$) differences across the spectrum of substrate concentration, spanning from 69 mg/L to 414 mg/L was observed. Depending on the genera, certain microbial species were reported to endure elevated paraquat concentrations (13, 27). Substrate concentration plays an essential role in paraquat degradation. The data obtained indicates that concentration above 276 mg/L was toxic to these bacterial strains, leading to proportional decrease in biodegradation efficiency of paraquat. Research by Wongputtisin et al., (28), reported the optimum substrate to be 0.5g/L with a similar findings reported also by (27, 29). This observation reinforces the notion that elevated paraquat concentrations could exert a toxic effect on metabolic activities, thereby potentially compromising the efficiency of bacterial degradation.

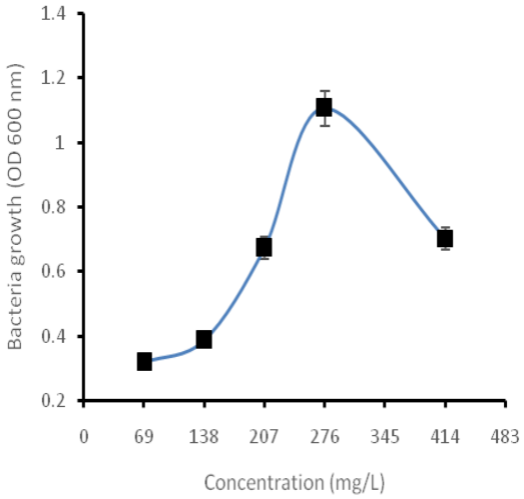

Fig. 4. Influence of various Paraquat Concentrations on the Growth of *Achromobacter sp. grown* on MSM after 48 h of Incubation at 37°C.

3.3.4 *Effect of Inoculum Size on Paraquat Degradation*

Figure 5 displayed the effects of varied inoculum sizes on the growth of paraquat-degrading bacteria within the MSM media. The growth of *Achromobacter* species strain BUK_BCH_TQ1 was found to be optimal at inoculum size of 200 µl (1 mL = 1.45 * 10^6 cfu), following 48 hours of incubation at 37 °C. A marked and significant ($p<0.05$) reduction in growth was observed when the inoculum size exceeds 300 µl. The decline in growth beyond the optimal inoculum size might be as a result of rapid increase in cell population, inducing competition for readily available nutrients that could lead to the death of less competitive cells. Alternatively, this phenomenon could arise due to insufficient dissolved oxygen available to cells and nutrient depletion.

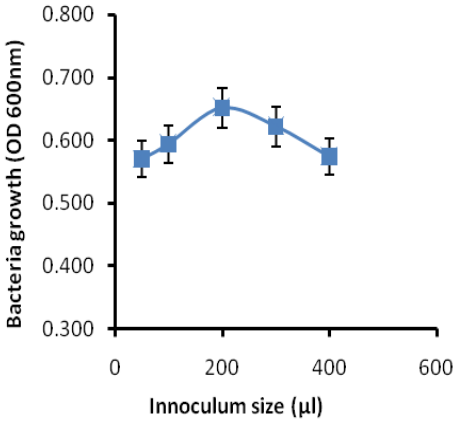

Fig. 5. Effect of Inoculum Sizes on *Achromobacterspg*rownin MSM Media incubated at 37 °C for 48 hours.

3.3.5 Effect of Temperature on Paraquat Degradation

Figure 6 illustrated the impact of different temperatures on the growth of paraquat-degrading bacteria in the MSM. From the graph, it was found that the optimal growth of *Achromobacter sp* strain BUK_BCH_TQ1 occurred at 35 °C after a 48-hour incubation period. Beyond this temperature threshold, a significant ($p<0.05$) reduction in growth was noted. Data analysis revealed a pronounced significance difference ($p<0.05$) across the range of temperatures; 25 °C, 30 °C, 35 °C, 40 °C, and 45 °C. Previous research has identified the optimal temperature range for paraquat degradation to be between 30-40°C, exemplified by the *Pseudomonas sp* (10). Elevated temperatures can influence the solubility and degradation processes of paraquat by bacteria. Specifically, higher temperatures can enhance paraquat solubility and bioavailability, while concurrently diminishing the solubility of oxygen, thereby impacting the activity of aerobic bacteria (13). Optimal growth conditions facilitate the secretion of an array of enzymes by microorganisms, which can effectively degrade the targeted toxic compounds. In contrast, unfavorable temperatures can hinder enzyme functionality either by obstructing substrate access due to lower temperatures or by affecting enzyme conformation.

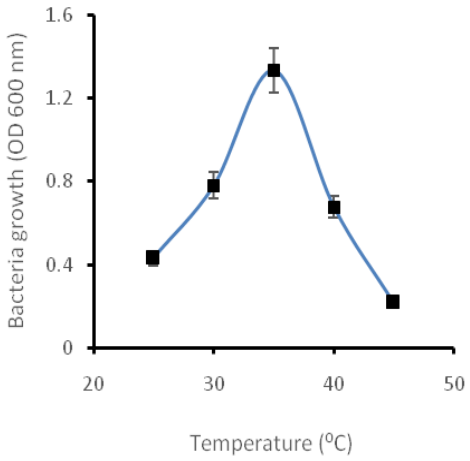

Fig. 6. Impact of Temperature on Paraquat-degrading *Achromobactersp* incubated for 48h.

3.4 Biodegradation Study

The degradation of paraquat was studied through HPLC analysis. Percentage (%) degradation by the isolated strain, sample together with control solutions were calculated and found that *Achromobacter sp.* degraded the compound by 91.01%.

$$(\%\ Degradation) = \frac{0.276 - 0.0248}{0.276} \times 100\% = 91.01\% \quad (1)$$

4. CONCLUSION

This study aimed at isolating and characterizing potential paraquat resisting bacteria through incubation experiment in mineral salt medium. A total of six bacterial isolates were tested out of which Achromobacter sp. strain BUK_BCH_TQ1 was chosen as the best following its 16S rRNA and biochemical characteristics.

The characterization activities were done using one factor at a time approach (OFAT) to determine the best conditions for its degradation.

The best conditions for the degradation of paraquat by *Achromobacter sp.* We`re achieved after 48 h incubation time, pH of 6.5, substrate concentration of 276 mg/L, 200 µl was determined to be the most conducive inoculum size, and a temperature of 35 °C.

Therefore, the isolate demonstrated the potential to biodegrade paraquat as a sole source of carbon and energy efficiently which could provide an eco-friendly approach for dealing with environmental pollution and toxicity caused by the herbicide. Hence, the isolate could be used for the biological degradation of paraquat which could serve as a potential tool for bioremediation of paraquat polluted sites.

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