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Isolation and Characterisation of Paraquat Degrading Bacteria from Agricultural Soil in Rigachikun Area of Kaduna Metropolis, Nigeria

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ABSTRACT

Paraquat is a compound that, in spite of its benefits, may produce a wide range of toxic side effects which pose a potential threat to the environment. This study was conducted to isolate and identify paraquat degrading bacteria from herbicide contaminated soil in Kaduna. The bacteria were isolated, identified and characterized using phenotypic characterization and Agarose gel electrophoresis. The agarose gel electrophoresis confirmed the presence of *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* in the soil obtained from the sample site. A concentration effect of 0.05%, 0.10%, 0.15%, 0.20% was observed and the initial and final cell mass was calculated. In microbial tolerance, it shows that 0.20% of paraquat in both *Bacillus subtilis* and *Staphylococcus aureus*, 0.10% of paraquat in *Escherichia coli* have the highest cell mass while 0.15%, 0.20%, 0.10% have the lowest cell mass respectively. Highest cell mass for each species shows the higher tolerance to paraquat, thus selected for biodegradation test. The spectrophotometric absorbance at 600nm wavelength was measured. *Bacillus subtilis* and *Escherichia coli* had an increase in absorbance for the first 48hours and later start decreasing while *staphylococcus aureus* had an increase in absorbance for the first 72hours then later started decreasing. *Bacillus subtilis* had the highest paraquat degrading capabilities, followed by *Escherichia coli* and *Staphylococcus aureus*. However, the optimal conditions for paraquat degradation have to be further studied to maximize the ability of the isolates.

Keywords: Bacteria, Biodegradation, Paraquat (1,1'-dimethyl-4,4'-bipyridylum dichloride), Soil

1.0 INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) is a broad-spectrum cationic contact herbicide that is widely used in more than 100 countries (Rashidipour *et al.*, 2019). Paraquat is applied against annual or perennial weeds of cotton, rice, soybean, and cocoa (Paraquat Information Center, 2018). It is widely used as non-selective contact herbicide (Almeida and Yonamine, 2007). Gramoxone is the most common trade name for paraquat. Farmers usually find it difficult to farm with hoe, so they use herbicide to overcome such challenge. Soil contains organisms such as bacteria that can be found in any natural system. Bacteria are the most abundant and predominant organisms in the soil. Soil would not be fertile without bacteria. These can be used to determine the biological availability of chemicals present in soil (Mahmood *et al.*, 2016). Pesticides are routinely used to prevent and control crop

diseases and pests, but pesticide residues have been linked to major health and environmental consequences. Because of the potential for runoff and leaching of these substances via the soil, contaminating surface and ground, extensive herbicide use has far-reaching implications (Wallis *et al.*, 2011). The two primary strategies for removing/reducing paraquat from the aquatic environments are currently adsorption and degradation. Previous research has found that activated carbon has a major role in paraquat adsorption (Kim *et al.*, 2016). Bioremediation of herbicide-contaminated soils has attracted a lot of interest in the scientific community (Zeng *et al.*, 2015). Microbial degradation is the safest means of checking pollution in the environment (Natala and Ochofe, 2009). By adding additives or increasing the availability of microorganisms, bioremediation techniques use living organisms to treat contaminated soil and water (Nie *et al.*, 2020). This

research has identified bacteria capable of degrading paraquat which is believed to be sustainable method for environmental bioremediation. The tolerance of bacteria and the capacity to degrade paraquat was determined in this research.

2.0 MATERIALS AND METHODS

2.1 Study Area

Rigachukun is a district in the Igabi Local Government Area of Kaduna state. Kaduna metropolis is the capital of Kaduna State and is located in the North-West geopolitical zone of Nigeria. It lies between latitude 10°20' N and 10.33° N, and longitude 7°45' E and 7.75°E (Mohammed *et al.*, 2015)

2.2 Collection of Soil Samples

Soil samples were collected from some agricultural lands in Rigachukun, while the control sample was collected from Kawo, Kaduna. Soil samples were collected at the depth of 30cm of the soil at the interval of 10 meters using a soil auger, placed in sterile polythene bags and taken to the laboratory for analysis.

2.3 Enrichment and Isolation of Paraquat Degraders

The soil enrichment medium method of Amakiri, (1982) was used. 150ml of liquid medium, 0.15ml paraquat was introduced into the liquid culture medium. The mixture was shaken thoroughly; 10g of soil sample was added and was incubated at room temperature ($30\pm 2^{\circ}\text{C}$) in an incubator shaker at 200rpm for 8days. After incubation, 0.1ml of the enriched broth was plated on nutrient agar and incubated for 5 days at room temperature. The bacterial isolates obtained were sub cultured to obtain pure colonies. The isolates were characterized and identified.

2.4 Identification of Isolates

2.4.1 Gram Stain

The method of Fawole and Oso, (2004) was used to identify the bacterial isolates and the morphology was studied

2.5 Molecular Characterization

2.5.1 DNA Extraction

The genomic DNA was obtained from 1ml of pure culture grown in nutrient broth using a Norgen DNA extraction kit (CAT. 61500).

2.5.2 Agarose Gel Electrophoresis

DNA electrophoresis in agarose gel was performed in buffer at pH 8.0. Tris-acetate was used. A UV transilluminator was used to inspect the DNA in the photograph.

2.5.3 Gel Imaging

The gel was put under a UV illuminated plate in a Biorad machine for viewing. The bands formed were captured in auto imaging system.

2.6 Microbial Tolerance of Different Paraquat Concentrations

The tolerance of concentration by microbial isolates was investigated. 20ml nutrient broth was dispensed into 50ml conical flasks in three replicates for each concentration. Different concentrations of paraquat viz 0.05%, 0.10%, 0.15% and 0.20% were separately added to the conical flasks in three replicates. A positive control flask was set up. The cell mass of the inoculum was determined on the first day before incubation at 27°C. The difference in cell mass of the inoculum was determined after five days interval. The tolerance of microbial isolates to paraquat was determined at the end of the incubation period. The species with the highest tolerance for each concentration was selected for biodegradation study.

2.7 Biodegradation Study

2.7.1 The Growth of Bacteria Resistant to Paraquat

The study of bacterial strains' ability to use paraquat as a sole carbon source was carried out in 250-mL Erlenmeyer flasks with 50 mL of mineral salt medium. For 120 hours, flasks were incubated at room temperature with orbital agitation (130 rpm). Under stationary culture, 1.0 mL of sample was collected at 24-hour intervals, 24, 48, 72, 96, and 120 hours to determine bacterial growth and paraquat degradation. Microbial growths were spectrophotometrically evaluated by measuring

absorbance at 600 nm. Varian 3400cx GC with a Hewlett Packard cross-linked methylsiloxane capillary column (12.5 m x 0.20 LD.) coupled with a Varian Saturn 2000 ion trap MS system was used to determine final residual (120 h) paraquat concentrations (Varian Inc., Walnut Creek, Calif.).

2.8 Data Analysis

The results obtained were analyzed using a T-test and a two-way analysis of variance (ANOVA) on an Excel statistical pack.

3.0 RESULT

3.1 Morphological Analysis of the Bacteria

The result for the gram stain reaction is presented in Table 1. It shows the isolates from the nutrient agar plate produced both gram-positive and gram-negative reactions.

Table 1: Morphological Analysis of Bacteria

Sample	Response to Gram
Plate	Sample on nutrient agar
A1	+ Long thread
A2	+ Long thread
B1	- Short thread
B2	- Short thread
C1	+ Cocci
C2	+ Cocci

KEY

+ = Gram positive reaction

- = gram negative reaction

3.2 Molecular Analysis

3.2.1 DNA Extraction

Replication of DNA using 16s rRNA sequencing

The genomic DNA isolates were amplified via PCR following the three-step protocol. The flanking site of ITS 1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4 (5' TCCTCCGCTTATTGATATGC 3') as forward and reverse primer were annealed and amplification of bacterial DNA was performed to know the 16s rRNA sequence which is highly conserved but with similar flanking regions.

3.2.2 Agarose Gel Electrophoresis

The plate shows the results with the agarose gel electrophoresis. It shows the relative molecular weight of the DNA bands. The band showed the DNA base pair for *Staphylococcus aureus* each of the DNA was 1500bp while that of *Escherichia coli* was 1400bp and that of *Bacillus subtilis* was 1420bp approximately.

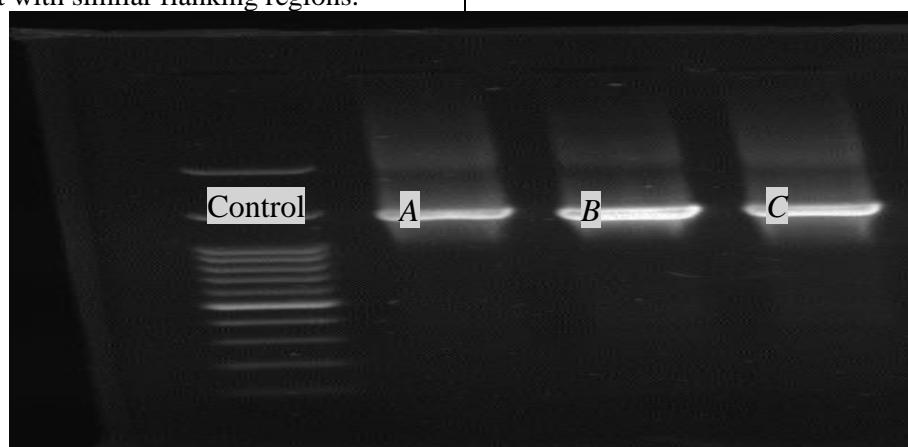


Plate 1: Agarose gel electrophoresis of *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*

KEY: A – *Bacillus subtilis*, B – *Escherichia coli*, C – *Staphylococcus aureus*

3.3 Microbial Tolerance of Different Paraquat Concentrations

The result for the microbial tolerance is presented in Table 2. It shows that 0.20% of paraquat in both *Bacillus subtilis* and *Staphylococcus aureus*,

0.10% of paraquat in *Escherichia coli* have the highest cell mass while 0.15%, 0.20%, 0.10% have the lowest cell mass respectively. Highest cell mass for each species shows the higher tolerance to paraquat, thus selected for biodegradation test.

Table 2: Microbial Tolerance of Paraquat Concentration

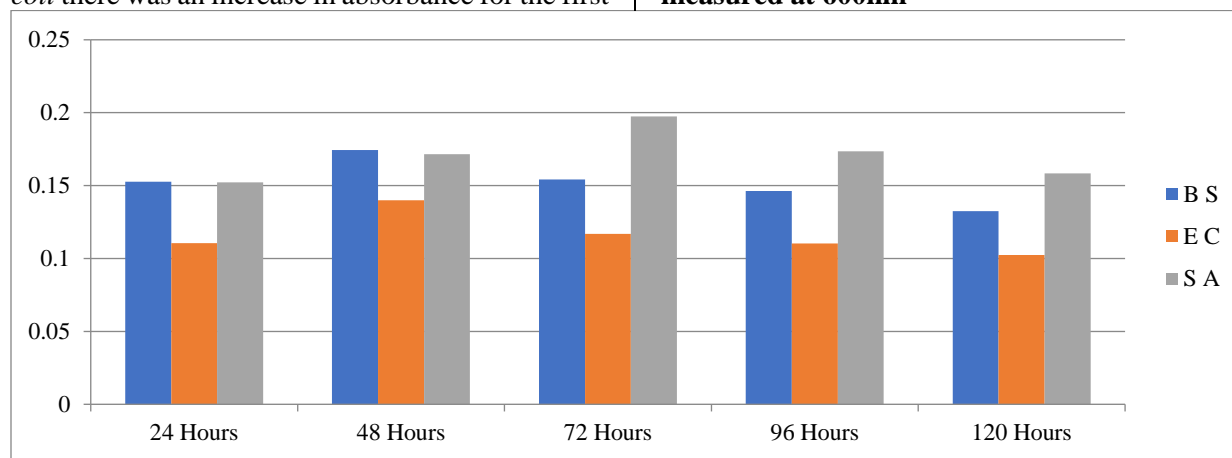
Paraquat Concentration (%)	Cell mass of Organisms		
	<i>Staphylococcus Aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia Coli</i>
0.05	98.09	99.12	94.17
0.10	96.12	98.91	98.19
0.15	97.54	97.64	98.09
0.20	98.90	99.16	86.92

3.4 Determination of biodegradation using the absorbance of the spectrophotometer

The result for biodegradation using the absorbance of the spectrophotometer is presented in the bar chart below. It shows the absorbance at 600nm wavelength. For *Bacillus subtilis* and *Escherichia coli* there was an increase in absorbance for the first

48hours and later start decreasing while for *staphylococcus aureus* there was an increase in absorbance for the first 72hours then later started decreasing.

3.5 Absorbance of the spectrophotometer measured at 600nm



KEY: B. S = *Bacillus subtilis*, E.C = *Escherichia coli*, S.A = *Staphylococcus aureus*

4.0 DISCUSSION

Isolation of bacterial strain through enrichment technique has been extensively used for the study of biodegradation of pesticides (Tatiane *et al.*, 2007). Based on the morphological and molecular characteristics, the bacteria identified in this study were *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. The bacteria identified morphologically are *Staphylococcus aureus* which was found in a similar study of Morphological and Biochemical identification of *Staphylococci* by

Mamza *et al.*, (2016) which occur in abundance with cocci shape. *Bacillus subtilis* which is by a study of isolation and screening of *Bacillus subtilis* by Madika *et al.*, (2017) and *Escherichia coli* which is in accordance with a recent study on isolation and identification of *E. coli* by Ali Saadi and Asmaa (2017) which are the dominant gram-positive bacteria with rod shape. Hence, the bacteria was identified and confirmed at the molecular level, with the expected bands of 1.4kbp obtained after PCR amplification of the 16S rRNA gene region of extracted bacterial genomic DNA

using a universal primer ITS 1 as forward primer and ITS 4 as reverse primer. In this study, the three organisms effectively degraded paraquat. *Bacillus subtilis* and *Escherichia coli* had an increase in absorbance for the first 48 hours and later started decreasing while *Staphylococcus aureus* had an increase in absorbance for the first 72 hours then later started decreasing. According to a study by Andy *et al.*, (2015), which compared fungi and bacteria degradation of paraquat suggest that bacteria constitute 20% of paraquat degraders compared to fungi. According to Ayansina and Oso (2008) reported that *Bacillus spp* are important in the degradation of many organic compounds. Within the first 5 days after incubation, the tolerance of different paraquat concentrations shows 0.20% paraquat concentration has the highest cell mass in *Bacillus subtilis* and *Staphylococcus aureus* whereas 0.10% paraquat concentration has the highest cell mass in *Escherichia coli*. In terms of lower cell mass, 0.15% of paraquat concentration has the lowest cell mass in *Bacillus subtilis*, whereas 0.10% has the lowest cell mass in *Staphylococcus aureus* and 0.20% of paraquat concentration has the lowest cell mass in *Escherichia coli*. In a survey by Naqvi *et al.*, (2011), the isolates' ability to use paraquat as their sole source of carbon and energy revealed that the isolates utilized paraquat at different rates. This indicates the varying capacity of microorganisms to degrade herbicide. The difference in paraquat degrading capability of the isolates may be a function of the difference in adhesion, cell membrane properties and/or the maintenance of their regimes in the test medium which was also stated by Nsikak and Aruwajoye (2011). Bacterial strains exhibited significant degradation ability and provided a potential tool for bioremediation of paraquat-contaminated environments. It is worth mentioning that in a patent filed by Li *et al.* (2017), a bacterial consortium achieved 97% paraquat degradation (100 mg/L). According to Jang *et al.*, (2002), it takes years for efficient degradation to occur. It was observed from this research that biodegradation depends on the characteristics, organism type and nature of parameters as reported by Shah *et al.* (2008) and conditions under which it takes place.

5.0 CONCLUSION

Paraquat-degrading bacteria with different potential were isolated from soil at Rigachikun. *Bacillus subtilis* has the highest degradation capacity and showed tolerance to the highest paraquat concentration. Thus, more research on *Bacillus subtilis* should be conducted to improve its tolerance to paraquat and its degrading capacity.

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