

Molecular Characterization of Bacterial that degrades herbicides isolated from soil environment in Abuja.

Owuna J.E¹, Asa A.A.², Ahmad A.A.¹, Ibrahim Y.¹, Haruna I.M.¹

¹Department of Microbiology, Nasarawa State University, Keffi, Nasarawa State, Nigeria.

²National Root Crop Research Institute, Umudike, Abia State, Nigeria.

Corresponding Author: ojodoyin@gmail.com

Received: 01 Oct 2021; Received in revised form: 28 Nov 2021; Accepted: 05 Dec 2021; Available online: 12 Dec 2021

©2021 The Author(s). Published by Infogain Publication. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract— The study was aimed to determine the molecular characterization of bacterial that degrades herbicides isolated from soil environment in Abuja. The systemic chemical herbicide was applied on an experimental plot of land with weeds and its effects on soil bacteria and the physicochemical properties of the soil was examined for a period of seven weeks. The chemical herbicide, glyphosate, reduced the plate count of bacteria from 120×10^5 cfu/g/dwt to 48×10^5 cfu/g/dwt some hours after application and the reduction continued till the end of the sampling period. The isolated bacterial species were *Simulium tani*, *Bacillus firmus*, *Pseudomonas tolaasii*, *Acinetobacter beijerinckii*, *Entrobacter sp*, *Citrobacter freundii*, *Pseudomonas poae*. Organisms that were eliminated following glyphosate application were *Bacillus magaterium*, *Pseudomonas tolaasii*, *Proteus sp*, and *Simulium tani* while those that persisted throughout the experiment were *Staphylococcus aureus*, *Pseudomonas poae*, *Bacillus firmus*, and *Entrobacter sp*. It was concluded that glyphosate altered the microbial counts and had a temporary inhibitory effect on the type of bacteria present in the soil.

Keywords—degrades, herbicide, soil, isolated, microbial count.

I. Introduction

Herbicides are valuable tools for the selective control of un-desirable plants in crop production. However, various herbicides at recommended rates, whether applied to the foliage or soil, often persist in the soil for extended periods of time. These residues may cause serious damage to sensitive plant species grown the season(s) following application of the herbicides. The climatic and edaphic factors, e.g., temperature, moisture, pH, soil composition, and cation exchange capacity which affect the residual life of herbicides, are numerous and complex.

Herbicides cause a range of health effects ranging from skin rashes to death. The pathway of attack can arise from intentional or unintentional direct consumption, improper Agricultural application resulting in the herbicide coming into direct contact with people or wildlife, inhalation of aerial sprays, or food consumption prior to the labeled pre-harvest interval. Pesticides can enter the human body through inhalation of aerosols, dust and vapour that contain pesticides, through oral exposure by consuming

food and water, and through dermal exposure by direct contact of pesticides with the skin (Cooper and Dobson 2007).

Herbicide are often applied directly to soil. They may also reach the soil through application to foliage via spray drift, run-off, or wash-off vectors. Once released to the environment, chemicals undergo various dissipation pathways, and the persistence of chemicals in the environment varies widely. Among factors affecting the local concentration of a compound are the amount of compound released, the rate of compound released, its persistence in the environment under various conditions, the extent of its dilution, its mobility, and the rate of biological or non-biological degradation (Ellis 2000 and Janssen *et al.* 2001).

Herbicide biodegradation involves a wide variety of microorganisms including bacteria and fungi operating under dynamic anaerobic and aerobic conditions. It is suggested that biodegradation of pesticides in soil ecosystems can only take place through the synergistic

interactions of a microbial consortium, the activity of which is affected by many soil physical and chemical properties, as well as the nature and extent of the pesticide contamination.

Soil microbes make valuable contribution to soil fertility. Pesticides can exhibit, stimulate and neutral effect on soil microbes, depending on the nature and concentrations as well as strain or types of microbes (Busse *et al.*, 2001).

Herbicides will remain toxic in soil when conditions are not favorable for microbes. Degradation of the herbicide follows the population growth of the microbes. During the lag phase the microbial population increases in response to food source and rapid decomposition occurs. (Busse *et al.*, 2001).

It drastically reduces the microbial population when applied to any soil sample. Synthetic herbicides have the potential to influence plant disease by several mechanisms. They can enhance disease or protect plants from pathogens due to direct effects on the microbe, to effects on the plant, or to effects on both organisms.

II. MATERIALS AND METHOD

Collection of soil samples

Soil samples were collected from the research farm of National Root Crop Research Institute, Nyanya Out Station, Abuja, Nigeria (Latitude 9.0765N and Longitude 7.3986E).

Soil samples were taken randomly with soil auger from each of the experimental plots and control plot, top soils of 0-15cm depth were used. Cassava and Maize are the crops grown in the farm. These crops have been sprayed with glyphosate organophosphorus herbicide for the last 4-5 years. Plots of land measuring 3x3m with four replicates arranged in randomized form was used for the experiment. Samples were collected before and after application of herbicide on from week one to the seventh week. Soil samples were sieved with 2.0mm mesh to remove stones and plant debris in soil. Samples were taken immediately to the laboratory into sampling bags for immediate analysis. (Makut and Ifeanyi 2017).

The herbicide, Glyphosate, was dissolved in distilled water at recommended rate of 50ml to five liter of water was used in this study, the mixture was then applied to the experimental plots and distilled water was added to the control plots for comparison.

Isolation of Bacteria from soil contaminated with herbicide.

One (1.0) gram of the soil sample was weighed using weighing balance suspended in 9ml of sterile water. It was properly mixed and a 10-fold serial dilution was carried out into seven dilutions.

The identification and characterization of bacterial isolates were based on cultural, morphological and biochemical characteristic using standard method. (Mendes *et al.*, 2017).

Molecular Identification of Bacteria isolated from herbicide contaminated soil.

The molecular identification of bacteria isolated from herbicide contaminated soil was carried out using Bacterial genomic DNA extraction, DNA quantification, 16SrRNA Amplification and Sequencing.

Determining the effects of Temperature, pH and Days on biodegradation of herbicides

Biodegradation experiment was carried out at two different temperatures pH and weeks (herbicides 3.0mg/ml) using the methods of Thavasi *et al.*, (2007).

Experiment to determine the effect of temperature on herbicides biodegradation by bacteria was carried out at various temperature for 15 days.

The effect of pH on biodegrading potential of bacteria was determined by adjusting the pH between pH4.5 and pH8.5 and were incubated for 15 days.

Effect of Days on herbicides biodegradation was carried out by incubating for different weeks ranging from 1- 7 weeks. (Jurado, et al, 2011).

Quantification of Pesticide Residue

This was carried out using Gas chromatography spectrophotometer on the biodegraded sample. The aqueous samples were analyzed by directly derivatizing an aliquot and the derivatizing reagent mixture was prepared fresh by mixing one volume of Heptafluoro-butanol to two volumes of Trifluoroacetic Anhydride.

III. RESULT AND DISCUSSION

The herbicide treatment used was observed to have negative effect on the microbial load. In glyphosate treated soil, there was a gradual decrease in bacterial population, that is, from 120×10^6 cfu first day after application to 101×10^6 cfu after one week of application. But by the third week of application, there was a sharp decrease of 77×10^6 cfu to 48×10^6 cfu by the sixth week of application. The bacteria count from contaminated and non-contaminated soil is as given in Table 1.

Table 1: Total Bacteria Counts for Bacteria 10^6 (cfu) /g/ dw.

SAMPLE	TIME OF SAMPLING	TBC	sampled	No. (%)	No. (%)	No. (%)
ES1	Immediately after herbicide application	120	<i>Pseudomonas poae</i>	5	0(0.0)	0(0.0)
CS1		132	<i>Pseudomonas tolaasii</i>	5	1(20.0)	0(0.0)
ES2	A week after herbicide application	101	<i>Proteus sp</i>	5	0(40.0)	1(60.0)
CS2		115	<i>Priestia flexa</i>	5	0(0.0)	1(20.0)
ES3	Two weeks after application	70	<i>Bacillus firmus</i>	5	0(0.0)	0(0.0)
CS3		110	<i>Bacillus magaterium</i>	5	1(20.0)	1(20.0)
ES4	Three weeks after application	75	<i>Simulium tani</i>	5	0(0.0)	1(20.0)
CS4		118	<i>Acinetobacter beijerinckii</i>	5	0(0.0)	0(0.0)
ES5	Four weeks after application	55	<i>Citrobacter freundii</i>	5	0(0.0)	0(.0)
CS5						
ES6	Five weeks after application	59				
CS6						
ES7	Six weeks after application	48				
CS7		97				

KEYS:

ES: Experimental Sample

CS: Control of Experimental Sample

TBC: Total Bacteria Count

Pseudomonas poae isolated from contaminated soil had percentage occurrence of 40.0% from Plot C and *Pseudomonas tolaasii* had 20.0% from plot A. *Proteus* sp had 60.0% occurrence from plot B, *Priestia flexa* had 20.0% occurrence from plot B, *Bacillus firmus* had 20.0% occurrence from plot C, similarly *Bacillus magaterium* had 20.0% occurrence from plot A and B respectively, *Simulium tani* had 20.0% occurrence from plot B, *Acinetobacter beijerinckii* and *Citrobacter freundii* had 20.0% occurrence from plot C respectively, Table 2.

Table 2: Percentage Occurrence of different Bacteria from Contaminated Soil with Herbicide

Bacteria	No.	Plot A	Plot B	Plot C
----------	-----	--------	--------	--------

The screening for survival of different bacteria in herbicides broth is as given in Table 3. The ability of the bacteria isolated from contaminated soil with herbicides showed that *Pseudomonas tolaasii*, *Pseudomonas poae*, *Proteus* sp, *Priestia flexa*, *Bacillus magaterium*, *Bacillus firmus*, *Simulium tani*, *Acinetobacter beijerinckii* and *Citrobacter freundii* were able to survival in herbicide concentration broth.

Table 3: Screening for Survival in Herbicides Broth

Bacteria	Lab code	Utilization
<i>Pseudomonas tolaasii</i>	Plot A 1a	+
<i>Pseudomonas poae</i>	Plot C 2b	+
<i>Pseudomonas sp</i>	Plot C 4a	-
<i>Proteus sp</i>	Plot A 1b	-
<i>Proteus sp</i>	Plot A 2a	-
<i>Proteus sp</i>	Plot B 5a	+
<i>Priestia flexa</i>	Plot A 3c	+
<i>Bacillus magaterium</i>	Plot B 4a	+
<i>Bacillus sp</i>	Plot B 5a	-
<i>Bacillus firmus</i>	Plot C 3c	+
<i>Simulium tani</i>	Plot B 2a	+
<i>Acinetobacter beijerinckii</i>	Plot C 4b	+
<i>Entrobactersp</i>	Plot A 5c	-
<i>Citrobacter freundii</i>	Plot C 1c	+

The effect of temperature on utilization of herbicide is as shown in Table 4. *Pseudomonas tolaasii* had the highest utilization at 35°C (2.19±0.26 mg/ml) followed by 30°C (2.06±0.64mg/ml) and least was at 26°C (1.23±0.1mg/ml).

Table 4: Effect of temperature on utilization of herbicides by different bacteria.

Isolates	Herbicide Conc. (mg/ml)	Temperature (°C)		
		26	30	35
<i>Pseudomonas tolaasii</i>	5	1.23±0.1	2.06±0.64	2.19±0.26
<i>Pseudomonas poae</i>	5	1.14±0.29	2.01±0.23	2.15±0.08
<i>Proteus</i> sp	5	0.53±0.86	1.97±0.05	1.92±0.16
<i>Priestia flexa</i>	5	1.55±0.15	2.12±0.19	1.94±0.34
<i>Bacillus magaterium</i>	5	1.48±0.24	2.00±0.03	1.80±0.05
<i>Bacillus firmus</i>	5	1.02±0.86	2.02±0.57	1.62±0.08
<i>Simulium tani</i>	5	1.12± 0.82	1.48±0.10	2.07±0.24
<i>Acinetobacter beijerinckii</i>	5	1.17±0.35	1.86±0.28	1.27±0.35
<i>Citrobacter freundii</i>	5	1.45±0.17	1.47±0.15	1.97±0.05

The effect of pH on herbicide utilization by bacteria isolates is as shown table 5. *Pseudomonas tolaasii* had the highest utilization at pH 7.0 (3.5±0.3mg/ml) followed by pH6.5 (3.1±0.3mg/ml), pH6.0 (2.1±0.1mg/ml) and the least was pH 5.5 (1.7±0.3mg/ml).

Table 5: Effect of pH on herbicide utilization by different bacteria isolates

Isolates	Herbicide Conc. (mg/ml)	Degree of Acidity and Alkaline (pH)			
		5.5	6.0	6.5	7.0
<i>Pseudomonas tolaasii</i>	5	1.7±0.3	2.1±0.1	3.1±0.3	3.5±0.3
<i>Pseudomonas poae</i>	5	2.0±0.1	1.9±0.8	2.1±0.3	2.8±0.8
<i>Proteus</i> sp	5	1.7±0.1	1.8±0.2	1.9±0.1	2.1±0.1
<i>Priestia flexa</i>	5	2.1±0.1	2.8±0.2	3.1±0.2	3.3±0.1
<i>Bacillus magaterium</i>	5	2.8±0.1	3.0±0.8	3.0±0.6	3.6±0.5
<i>Bacillus firmus</i>	5	2.3±0.2	2.6±0.1	2.8±0.2	2.8±0.3
<i>Simulium tani</i>	5	2.6±0.8	2.8±0.1	2.8±0.2	3.0±0.1
<i>Acinetobacter beijerinckii</i>	5	1.7±0.2	1.8±0.2	2.0±0.5	2.6±0.1
<i>Citrobacter freundii</i>	5	1.6±0.1	1.8±0.2	2.0±0.5	2.8±0.2

DNA sequence analysis/molecular identification of microbes

Blast analysis of the gene sequence of the pure bacteria culture identified three bacteria species of the genus *Pseudomonas*, *Priestia* and *Bacillus*, of which *Pseudomonas* dominated the samples, Table 6.

The relatively high abundance of *Pseudomonas* species in the samples might be due to their high ability to tolerate

and degrade pesticides (Darsaet, al. 2014). Similar studies have been conducted by Asef 2014 and have documented the isolation, molecular characterization and pesticide degradation by *Aspergillus* species. Therefore, the presence of these bacterial species in our study can suggest their biodegradation potential towards pesticide.

Table 6: Molecular Characterization of Bacterial Isolates

Sample ID	Organism Identified by BLAST	Identity (%)	Sequence Length (Bp)
Plot A 1a	<i>Pseudomonas tolaasii</i> strain Pt11 <i>Pseudomonas sp.</i> bs2935 <i>Pseudomonas sp.</i> MYb193 <i>Pseudomonas libanensis</i> strain DMSP-1	88	6475196
Plot C 2b	<i>Pseudomonas poae</i> strain PMA22 <i>Pseudomonas antarctica</i> strain BS2772 <i>Pseudomonas sp.</i> ADAK22 <i>Pseudomonas lurida</i> strain MYb11	90	6530734
Plot B 4a	<i>Bacillus magaterium</i> strain PHB06 <i>Bacillus sp.</i> strain magaterium-M1 <i>Bacillus sp.</i> strain AM136 <i>Priestia magaterium</i> strain R2A90	100	1493
Plot A 3c	<i>Priestia flexa</i> strain QG-3 <i>Priestia flexa</i> strain TH25 <i>Priestia flexa</i> strain FYF01 <i>Priestia flexa</i> strain BUMD13	100	1515
Plot C 3c	<i>Bacillus firmus</i> <i>Bacillus sp</i> mixed culture X3-37 <i>Bacillus sp</i> Al-Dhabi-17 BAU <i>Bacillus sp</i> strain 35-Lb11/2	95	1359
Plot B 2a	<i>Simulium tani</i> Uncultured bacterium clone MSD18_A02 <i>Bacillus sp.</i> mixed culture X3-37 <i>Bacillus sp.</i> Al-Dhabi-17	100	1420
Plot C 4b	<i>Acinetobacter beijerinckii</i> strain LMA2 Uncultured <i>Priestia sp</i> clone RJCEP_01 <i>Priestia aryabhattai</i> strain MSAR20 Uncultured actinobacterium clone STJ C42	88	786
Plot C 1c	<i>Citrobacter freundii</i> strain RHBSTW <i>Enterobacter sp.</i> RHBSTW-00975 <i>Enterobacterasburiae</i> MRY18-106 <i>Enterobacter sp.</i> HP19	100	109959

Phylogenetic analysis

The Phylogenetic analysis revealed that the soil contained diverse bacterial clustering into three orthologous groups. Reason maybe the combination of selective factors,

proximity and functional capacity of microbes. Functionally, phylogenetically distant lineages can share common functional features and functions (Ning and Beiko 2015). Ning and Beiko 2015 also opined that functional similarities exist between operational taxonomic units (OTUs) that belong to different high-level taxonomic groups. Most of microbial sequences analyzed in different taxonomic divisions could be related to representatives with known metabolic traits.

Correlation between different parameters

Some correlations were also calculated from the results, at the end of the experiment when the organisms were suggested to be highly metabolic active. The negative correlation observed between pesticide degradation and colony count suggests the negative impact pollutants may have on biodiversity. These relationships would be useful to biodegradation of glyphosate and other organic contaminants in the environment (Showunmied, al. 2020).

IV. CONCLUSION

From the findings of this study, it suggests that the organisms isolated and identified have the potential to degrade glyphosate pollutants when applied in the environmentally friendly technology clean-up (bioremediation) of glyphosate contaminated environment. Therefore factors promoting their growth should be encouraged.

Herbicides are phytotoxic chemicals used for destroying various weeds or inhibiting their growth. It is important to also know that excess use of herbicides in agroecosystems may change composition of weed populations and diversity.

Excess use of herbicide should be minimize in wildlands, as herbicides may increase the diversity of native species. Threats to plant biodiversity caused by habitat loss and invasive species are far greater than threats by use of herbicides.

It is also important to properly managed lands that are spread with herbicide as spray runoff in sandy soils may cause tree injury if followed soon after with irrigation or rainfall.

To prevent contamination of water bodies, management plans should carefully consider the hydrology of the system that is being treated. Hypothesize potential runoff scenarios and take appropriate measures (such as buffer zones) to prevent them. Underground aquifers and streams should be considered as well.

- [1] Asef, I. M. Isolation of pesticide degrading microorganisms from soil. *Advances in Bioresearch* 2014; 5(4): 164-168.
- [2] Busse, M.D., Ratcliff, A.W., Stestak, C.J., Powers, R.F. (2001). Glyphosate toxicity and the effects of long term vegetation control on soil microbial communities. *Soil Biol. And Biochemistry J.* Vol.33:1777-1789.
- [3] Cooper, J. and Dobson, H. 2007. The benefits of pesticides to mankind and the environment. *Crop Protection* 26(9): 1337-1348.
- [4] Darsa, K., Thatheyus, J. and Ramya, D. 2014. Biodegradation of petroleum compound using the bacterium *Bacillus subtilis*. *Science International*. 2014; 2: 20-25.
- [5] Ellis, F. (2000). The determinants of rural livelihood diversification in developing countries. *J. Agricultural Economis*. 51, 289-302.
- [6] Janssen, M.A. (2007). An update on the scholarly networks on Resilience, Vulnerability and Adaptation within the human dimensions of global environmental change. *J. Ecology and Society*. 12(2):9-15.
- [7] Jurado, A.S., Fernandes, M.A.S., Videira, R.A., Peixoto, F.P., Vicente, J.A.F. (2011). Herbicides: The Face and the Reverse of the Coin. An in vitro Approach to the Toxicity of Herbicides in Non-Target Organisms. In: KORTEKAMP, A. (Ed.) *Herbicides and Environment*. Croatia, 3- 45.
- [8] Makut M.D. and Ifeanyi U. E. (2017). Screening for citric acid producing fungi from the soil Environment of Jos North, Plateau State, Nigeria. *FULafia Journal of Science & Technology*. 3 (1):1-12
- [9] Mendes, K.F., Freguglia, R.M.O., Martins, B.A.B, Dias R.C., Pimpinato R. and Tornisiello V.L (2017). Cow bonechar for pesticide removal from drinking water. *Scholars Journal of Agriculture and Veterinary Sciences*. 4:504-512.
- [10] Ning, J. and Beiko, G. 2015. Phylogenetic approaches to microbial community classification. *Microbiome* 2015; 3(1): 47-49.
- [11] Sowunmi, K. Shoga, S.M. Adewunmi, O.M. Oriyomi, A.F. Sowunmi, L. 2020. Isolation and Molecular Characterization of Pesticide Degrading Bacteria in Polluted Soil. doi: <https://doi.org/10.1101/2020.07.21.213942>.
- [12] Thavasi, R., Jayalakshmi, S., Balasubramanian T., Banat, I.M. (2007). Effect of salinity, temperature, pH and crude oil concentration on biodegradation of crude oil by *Pseudomonas aeruginosa*. Retrieved from www.acikenisim.uludag.edu.tr on Jan 12, 2021.

REFERENCES