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Histoarchitecture of albino rat's liver

Isolation of Organophosphate (Chlorpyrifos)-degrading Bacterium from Agricultural Farmlands in Batagarawa Local Government Area of Katsina State

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ABSTRACT

Chlorpyrifos being the most frequently used organophosphorus pesticide is a broad-spectrum insecticide The massive application of chlorpyrifos has led to the contamination of water and soil which disrupt the environmental quality as many of its derivatives were detected in various ecosystems. This study is to isolate chlorpyrifos-degrading bacteria from agricultural farmland in Batagarawa Local Government area of Katsina State. Two chlorpyrifos-degrading bacteria were isolated using dilution technique followed by selective enrichment on minimal medium with chlorpyrifos as sole carbon source from soil samples collected from an agricultural farmland in Batagarawa Local Government Area of Katsina State. The isolates were characterized by Gram staining and biochemical test using Vitek II automated machine. The strains A10, A12 and A13 were identified to be *Pseudomonas* spp. and *Staphylococcus* spp.

INTRODUCTION

Pesticides and their byproducts are a significant ecological issue that leads to environmental pollution [1]. Studies have shown that organophosphate pesticides make up the largest group of pesticides worldwide, representing 38% of total pesticide usage [2]. Chlorpyrifos, the predominant organophosphorus pesticide, is a highly effective insecticide that targets a wide range of insects [3]. The extensive use of chlorpyrifos has resulted in the pollution of water and soil, causing a disturbance in environmental quality due to the presence of its various derivatives in different ecosystems [4,5]. Hence, it is crucial to promptly identify and cleanse the contaminated environment that contains chlorpyrifos. Pesticides are essential for plant production, particularly for economically significant crops, as they safeguard one-third of the global agricultural products [6]. Approximately two million metric tons of pesticides are utilized worldwide, with herbicides comprising 47.5%, insecticides accounting for 29.5%, fungicides making up 1.5%, and the remaining 5.5% being classified as other types of pesticides [7]. Currently, there are 140 organophosphate compounds used as plant growth regulators around the world which are components of more than 100 types of commercially available pesticides such as chlorpyrifos, paraoxon, parathion, dichlorvos and Diazinon [7]. Biodegradation using autochthonous microorganisms for pesticide removal from the environment is quite attractive [9]. The success or failure of bioremediation depends on several factors, such as the competitive ability of the remedial agents, availability of pollutants, and abiotic factors [8]. Microbes have the ability to transform or degrade xenobiotics [9,10].

A pesticide refers to a combination of chemicals designed to protect crops from being damaged by insects, mites, nematodes, weeds, or rats [11]. These chemicals can pose risks to public health during their production, processing, storage, transport, or marketing [12]. Pesticides can be categorized based on the specific organism they are designed to target, such as herbicides for plants, insecticides for insects, nematicides for nematodes, molluscicides for mollusks, and rodenticides for rodents [13].

Organophosphates are utilized for the management of a diverse range of insects that feed by sucking, chewing, or boring, as well as spider mites, aphids, and crop-damaging pests [14]. All organophosphorus pesticides are esters of phosphoric acid, which include derivatives of aliphatic, phenyl, and heterocyclic compounds. Due to the widespread and continuous use of Organophosphate compounds, there have been reports of soil and water systems being contaminated globally. Environmental microorganisms utilize Organophosphate compounds as a source of both phosphorus and carbon, effectively breaking them down. According to a study, more than 98% of the insecticides that are sprayed and 95% of the herbicides end up in places other than where they were intended to go [15].

Pesticides become airborne as they are dispersed by wind, thereby potentially polluting other regions through contamination. Pesticides have the potential to induce water pollution, as certain types of pesticides are long-lasting pollutants that can contaminate both soil and water sources [16]. Pesticides diminish biodiversity, exacerbate the decline of pollinators, devastate habitats, particularly those of avian species, and pose a threat to endangered species [17]. Pests have the ability to acquire resistance to pesticides, which requires the development of a new pesticide. Alternatively, a higher concentration of the pesticide can be employed to combat the resistance, albeit at the expense of exacerbating the issue of environmental pollution [18].

EXPERIMENTAL METHOD

Sample Location

Different sampling stations were identified during the study. The selection of sampling sites was based on the frequent usage of organophosphate pesticides on farmland during agricultural activities in Batagarawa, the local Katsina State government.
Soil samples from the organophosphate pesticide-contaminated sites were collected within 0-10cm surfaced depth. All samples were collected after clearing the surface litter and transported within a few hours of collection to the microbiology laboratory Umaru Musa Yaradua University (UMYU), Katsina, for processing.

Bacteria identification

Preliminary bacteria identification of organophosphate was carried out using morphology and relevant biochemical tests. Gram staining was conducted on each screened isolate, differentiating Gram-positive and negative organisms [22,23].

Biochemical tests of the identified bacteria using Vitek automated machine

Vitek automated system was used to characterize the obtained bacteria isolates biochemically. This was done by turbidimetry-controlled suspension of pure colonies in saline, which were inoculated into the identification cards containing 64 different broths and one negative control card. By measuring light attenuation with an optical scanner, the Vitek-programmed computer determines whether each well is positive or negative. When the incubation period was complete, the reactions were analyzed automatically, and the identification result was printed.

RESULTS AND DISCUSSION

Bacteria Isolation and Screening

A total of sixteen different bacteria strains coded A01 to A16 were isolated from Hassan Usman Polytechnic and Batagarawa farms with varying colony counts (**Table 2**). The screening based on different concentrations of organophosphate indicated strains A10, A12, and A13 as the best organisms that tolerated up to 0.3% v/v in MSM with 0.7, 1.53, and 1.83 x 10^2 cfu/g colony counts, respectively (**Table 2**). Dilys [19] reported that his table is similar to this research, where he works on isolating bacteria capable of degrading chlorpyrifos from agricultural soil at Amansea, Anambra State, Nigeria.

Table 1. Bacteria isolated from the contaminated soil within Katsina and Batagarawa**.**

Strain code	Sampling sites	Coordinates	Colony counts ± STD (x10^2 CFU/g)
A01	HUPK farm	12⁰55'51.55"N; 7⁰36'21.70"E	0.30±0.08
A02	HUPK farm	12⁰55'51.42"N 7°36'21.53"E	0.31±0.13
A03	Batagarawa farm	12⁰ 55'2.86"N 7⁰36'15.88"E	0.27±0.06
A04	Batagarawa farm	12⁰ 55'2.82"N 7⁰36'15.57"E	0.20±0.08
A05	HUPK farm	12⁰55'51.72"N 7⁰36'21.85"E	0.20±0.05
A06	Batagarawa farm	12⁰ 55'2.79"N 7⁰36'15.60"E	0.19±0.08
A07	HUPK farm	12⁰55'51.45"N 7⁰36'21.81"E	0.33±0.19
A08	HUPK farm	12⁰55'51.73"N 7⁰36'21.68"E	0.29±0.17
A09	Batagarawa Farm	12⁰ 55'2.54"N s7⁰36'15."E	0.22±0.07
A10	HUPK farm	12⁰55'51.35"N 7⁰36'21.63"E	0.95±0.08
A11	HUPK farm	12⁰55'51.65"N 7⁰36'21.52"E	0.45±0.09
A12	HUPK farm	12⁰55'51.55"N 7⁰36'21.70"E	1.06±0.25
A13	Batagarawa Farm	12⁰ 55'2.89"N 7⁰36'15.81"E	1.17±0.30
A14	Batagarawa Farm	12⁰ 55'2.34"N 7⁰36'15.72"E	0.41±0.17
A15	HUPK farm	12⁰55'51.75"N 7⁰36'21.68"E	0.32±0.14
A16	Batagarawa Farm	12⁰ 55'2.77"N 7⁰36'15.74"E	0.39±0.19

Note: HUPK means Hassan Usman Polytechnic Katsina; A01 to A16 are the codes of individual bacteria isolated

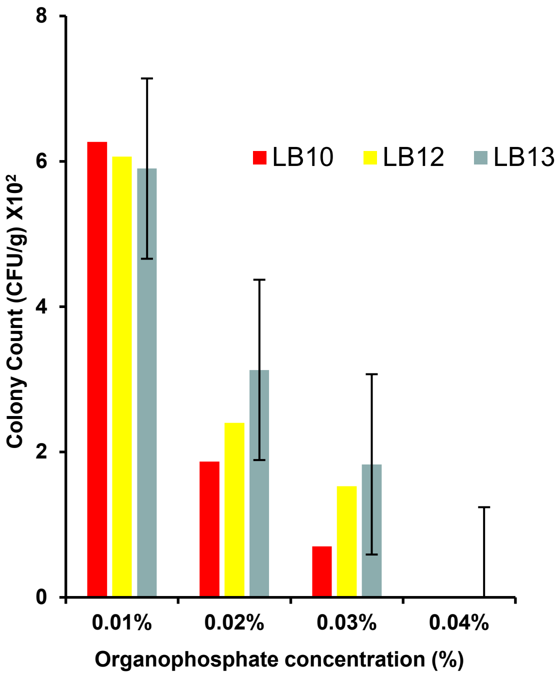

Fig. 1. Screening of bacteria isolates during biodegradation of organophosphate pesticide at varying concentrations based on the colony-forming units in MSM agar.

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Identification of the screened bacteria strains A10, A12 and A13

Based on the result obtained from the Vitek analyses, more than 40 biochemical tests were conducted, and isolates were found to possess similar characteristics as those of the *Staphylococcus lentus, Pseudomonas aeruginosa* and another *Staphylococcus lentus* for the A10, A12, and A13 respectively. The similarities were more than 95%, and therefore the strains were confirmed to be those identified bacteria considering the additional colony and microscopic morphologies. A total of three (3) chlorpyrifos degrading bacteria were finally screened from the agricultural soil collected at Batagarawa Local government area of Katsina state, Nigeria. The bacteria were identified as *Staphylococcus* and *Pseudomonas* species using Vitak automated identification. The results obtained in this study were in line with that of Yadav *et al*. (2015), who isolated *Staphylococcus*, *Micrococcus*, *Enterobacter*, *Bordetella*, *Pseudomonas,* and *Klebsiella* species from Agriculture University Gwalior, India. A similar report was conducted by Omeiri et al. (2024) and that of Wepukhulu [21], who worked on the biodegradation of chlorpyrifos by bacterial strains isolated from Lebanese soil and its association with plant growth improvement—optimization of Growth Conditions for Chlorpyrifos-Degrading Bacteria in Farm Soils in Nakuru County, Kenya.

CONCLUSION

This study successfully identified and characterized bacterial strains capable of degrading organophosphate pesticides in agricultural soils from Batagarawa, Katsina State, Nigeria. A systematic sampling of contaminated soils followed by microbial isolation and identification revealed three highly effective chlorpyrifos-degrading bacteria: *Staphylococcus lentus* and *Pseudomonas aeruginosa*. These strains demonstrated significant tolerance and biodegradation efficiency at varying concentrations of organophosphate pesticides, confirming their potential for bioremediation applications. The findings align with previous studies. The use of Vitek automated systems for detailed biochemical characterization further validated the identification and efficacy of the isolated strains. Overall, the study underscores the importance of microbial diversity in contaminated soils and its potential application in sustainable agricultural practices, particularly for the bioremediation of pesticide residues. Future research should focus on optimizing growth conditions and understanding the genetic and metabolic pathways involved in pesticide degradation to enhance the efficiency and applicability of these bioremediating bacteria in different environmental contexts.

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