

Isolation, Characterization, and Biodegradation Potential of *Bacillus* Species on Selected Pesticides in Agricultural Soils of Enugu State, Nigeria

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Abstract

This study investigates the isolation, characterization, and evaluation of Bacillus species in the biodegradation of selected pesticides. Soil samples were collected from agricultural farmlands in Amechi and Agbani, Enugu State, Nigeria, where pesticide application is a common practice. The pesticides used in this study include Ridoff (organophosphate-based) and Lara Force (pyrethroid-based). Microorganisms capable of utilizing these pesticides were isolated using mineral salt medium and further identified through biochemical characterization techniques such as Gram staining, motility test, spore staining, catalase, oxidase, and indole tests. The results revealed that the dominant isolates were Bacillus subtilis and Bacillus licheniformis. These isolates demonstrated significant potential in degrading both pesticides over a 21-day incubation period. Degradation efficiency was assessed using spectrophotometric analysis and colony count methods. Lara Force showed a higher degradation rate compared to Ridoff, with values ranging from 0.336 to 1.848, while Ridoff recorded values between 0.600 and 1.731. Environmental factors such as temperature and incubation time influenced degradation efficiency. The findings of this study highlight the effectiveness of Bacillus species in the biodegradation of pesticide residues, suggesting their potential application in bioremediation strategies for contaminated agricultural soils. This approach offers an ecofriendly and cost-effective alternative to chemical methods of pollution control.

Introduction

Most pesticides are synthetic, organic chemicals which are classified and characterized in terms of the type of pest they target (Garud et al, 2024). The four major pesticides group include herbicides, insecticides, fungicides and nematicides, are designed for the control of weed, insects, plant pathogenic fungi and nematodes respectively (Pathak et al., 2022) According to Ifediegwu *et al.*, (2015), pesticide application on agricultural soil is now a common practice and is an important factor of integrated pest management (IPM) strategies. Some of these pesticides persist in the soil to form pollutants which may occasionally lead to surface and groundwater contamination. Pesticides have a great impact on human health protection and preservation of food fibres and other cash crops by controlling disease vectors and by keeping in check many species of unwanted insects and plants. More than 55% of the land used for agricultural production in developing countries uses about 26% of the total pesticides produced in the world (Food and Agriculture Organization [FAO], 2023).

The excessive use of pesticides leads to an accumulation of a huge amount of pesticide residue in the food chain and drinking water environment that further leads to a substantial health hazard for the current and future generations due to the uptake and accumulation of these toxic compounds (Guerrero et al., 2023). Degradation of pesticides may occur through photodegradation, chemical degradation or biodegradation (Bhagawati et al., 2020). Biodegradation can be defined as any process that uses microorganisms (Bacteria, Fungi), green plants or their enzymes to return the natural environment altered by contaminant of the original condition (Salam et al., 2024). The use of microbes in the bioremediation and detoxification of many toxic xenobiotics, especially toxic pesticide is an efficient tool for the remediation of contaminated sites in the environment (Guerrero et al., 2023). Abraham and Silambarasan (2016) isolated an *Enterobacter* strain capable of degrading chlorpyrifos. Similarly, Deng et al. (2015) and Zhao et al. (2017) isolated *Stenotrophomonas* and *Sphingomonas* species, respectively, which could utilize chlorpyrifos as a primary source of carbon and phosphorus for growth. Microorganisms possess many enzymes to degrade organochlorine pesticides through reductive dichlorination, dehydrochlorination, oxidation and isomerization of the parent molecules (Srivastava, 2026). Soil type, soil pH, soil temperature, soil moisture content, organic matter contents, and microbial populations are considered to be among the most important environmental factors influencing pesticide degradation in soil (Yasir, 2025). Many microorganisms that are able to degrade organophosphate pesticides have been isolated from soil around the world. Previous research has shown that pesticide Malathion, Chlorochlorpyrifos, Dichlorvos degrading bacteria applied as single strains or as consortia to increase the rate of degradation of pesticides such as Malathion, Chlorochlorpyrifos, Dichlorvos in soil (Dar & Kaushik, 2023). Degradation of pesticides is usually a combination of a number of processes, including microbial degradation and chemical hydrolysis and is also influenced by some physiochemical properties such as temperature, pH and carbon and nitrogen source (Yasir, 2025). However, biodegradation is the primary mechanism of pesticide degradation and detoxification in soils. Thus, bacteria and other microbes may have a major effect on the persistence of most organophosphate pesticides in soil (Dhakal, 2025). Biodegradation is a common method for the removal of organophosphate pesticides because of its low cost and low collateral destruction of indigenous animal and plant organisms in health of the environment (Srivastava, 2026). The biological strategies where microorganisms are used to act upon these pesticides remains the most efficient and cost-effective option to clean up pesticide-contaminated sites. Such types of microorganisms generally have diverse ability to act upon the pesticides including their ability to degrade them in to non-toxic compounds (Srivastava, 2026). Various bacteria having the ability to degrade carbamate group of pesticides have been isolated from agricultural lands having a history of pesticide usage (Dhakal, 2025). Presence of pesticides in agricultural soil signifies the beginning of bioremediation for pesticides-degrading microorganisms. Therefore, this study aims to isolate possible pesticide-degrading bacillus species, identify bacterial isolates and evaluate their efficiency in bioremediation.

Materials and Methods

Sample collection:

The soil samples that were used in this study were collected using two (2) sterile sample collection bottles from two (2) rice farm land. The farmlands are situated at Amechi and Agbani, both of which are situated in Awkunanaw, Enugu State, Nigeria. These farmlands were said to produce food crops for commercial purpose and the application of pesticides were paramount in order to achieve desired harvest by reducing and removing pests that causes plant diseases.

The pesticides used in this study are Ridoff and Lara Force which is composed of organophosphate and pyrethroid compounds respectively. They were bought from a chemical dealing shop at Okpara Avenue, Enugu State, Nigeria.

Sample Preparation:

After the sample collection, the soil samples were sieved to remove debris and stones using spatula and to also make the soil appear in fine texture. 10g of the soil samples were weighed out each from the two soil samples using an electric weighing balance into 250ml Erleimeyer bottle. 0.1ml from each of the pesticides were taken and diluted into 100ml of water.

Media used and their preparation:

Mineral salt medium (broth) was used in the inoculation of the soil samples and the pesticides. 100ml into four places of the medium were prepared. Mineral Salt Medium (MSM) used was of the following composition:

- NH_4NO_3 , 2g; K_2HPO_4 , 0.06g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2g; Yeast extracts, 2g; NaCl, 0.2g; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.2g; Agar, 3g.

Nutrient agar was prepared and used for the maintenance of culture, 2.8g for 100ml.

Inoculation and Incubation:

Each 100ml of the four media prepared were mixed with 1ml of the organophosphate and 1ml of pyrethroid which were taken from the 100ml of water with 0.1ml of the pesticides, each into the four conical flasks containing the soil samples. The media along with the samples were agitated and shaken 20 times and were incubated at room temperature of 26.6°C – 27°C for 3 days.

Two loopfuls of the organisms that grew on the medium of the initial samples were taken and inoculated into a fresh mineral salt agar (MSA) and incubated for 24hrs.

After 24hrs, 2 loopful of the organisms that grew on the MSA were again inoculated into mineral salt medium (broth) containing 1ml of the pesticides for 3days. This is done to confirm the pesticide-utilizing capacity of the microorganism.

Then from the results of the previous stage of the organisms were inoculated into mineral salt agar and also into Nutrient Agar. The incubation lasted for 48hrs on MSA but 24hrs on Nutrient Agar before growth was noticed.

Degradation determination

Mineral Salt Medium (100g) was prepared into different conical flasks containing 1ml and 5ml of each test pesticides and loopfuls of the organisms from the result of the previous stage were inoculated for 24hrs at room temperature of 26°C .

After 24hrs, I started checking for the degree of degradation using spectrophotometer at wave length of 540nm. Colony count was carried out using serial dilution and concentration of 10^{-3} , 10^{-4} and 10^{-5} were used. 0.1ml of each concentration was inoculated using spreader method.

Effect of temperature on the degradation of Pyrethroid and Organophosphorus pesticides

In order to determine the optimal temperature for efficient degradation of pyrethroid and organophosphate, the MSM broth was placed for incubation at various temperatures (30°C , 26.8°C , 26.5°C , 26.5°C , 28°C and 27°C) for Day 1, Day 4, Day

5, Day 7, Day 14 and Day 21 respectively.

Biochemical Characterization of *Bacillus* spp

Gram stain:

Gram's Stain is one of the most frequently used processes in identifying bacteria. It is a primary test which quickly and cost effectively divides bacteria into two (2) types: Gram-Positive or Gram-Negative.

Gram stain was carried out on the isolated organisms to test the organisms for gram reactions. One loopful of each of the organisms that were isolated from the different sample soils were placed and smeared on microscopic slides with a sterile wire loop. The organisms were allowed to air dry and afterwards were heat fixed. 5 drops of crystal violet were added on the organisms and were allowed to stand for 1 minute and were washed off with water afterwards. 5 drops of iodine solution were then added and allowed to stand for 30 sec and washed briefly with water. The slides were then tilted and alcohol was used to run on the organisms to decolorize the purple color. They were immediately washed with water for 5 seconds. 5 drops of safranin were added and were left to stand for 1 minute and were briefly washed off with water. The organisms on the various slides were then examined under the microscope at 400x and 1,000x oil immersion. One examination result showed that the organisms retained the purple color of crystal violet proving that the organisms are gram positive.

Motility Test

Motile bacteria move about with structures called flagella (a few exceptional bacteria move the help of axial filaments, which cannot be seen in the microscope). In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye. Loopful of the organisms each were taken from fresh or young subculture plates from each of the samples. The organisms were mixed with a drop of sterile water at one end of a clean glass slide and at the other end of the slide, the organisms were mixed with a drop of peptone water. At each end of the slides were covered with cover glass. The slides were then examined under the microscope at x40 objective lens.

Spore Staining

Thin film of *Bacillus* colonies was made on glass slide. The film was flooded with Malachite green. It was heated gently, at almost 3-5 mins. Then the slide was counterstained with safranin, dried and viewed with oil immersion lens at x100. The stained slides were examined under the microscope for the presence of spores. The spores in the organisms were bright green.

In the Schaeffer-Fulton's method, a primary stain-Malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolorized with water. Safranin is then applied to counterstain any cells which have been decolorized. At the end of the staining process, vegetative cells will be pink, and endospores will be dark green.

Spores may be located in the middle of the cell, at the end of the cell, or between the end and middle of the cell. Spore shape may also be of diagnostic use. Spores may be spherical or elliptical.

Catalase Test

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2 . The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the catalase enzyme.

Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water. To find out if a particular bacteria isolate is able to produce catalase enzyme, small inoculum of bacterial isolate is mixed into hydrogen peroxide solution (3%) and the rapid elaboration of oxygen bubbles occur. The lack of catalase is evident by a lack of weak bubble production.

One loopful of the organisms were transferred to a clean glass slides using sterile wire loop. Two drops of 3% hydrogen peroxide (H_2O_2) on the slide and were mixed. Positive results were taken note of by the production of bubbles.

Indole Test

Indole test is used to determine the presence of the enzyme tryptophanase. Tryptophanase breaks down tryptophan to release indole, which when reacted with cinnamaldehyde produces a blue-green compound. The absence of the enzyme results in no color production (i.e. indole negative). 1% paradimethylaminocinnamaldehyde reagent was saturated on pieces of filter papers. Wooden sticks were used to collect the colonies of the test organisms and rubbed on the filter paper.

Oxidase Test

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless.

Filter papers were soaked with tetramethyl-p-phenylenediaminedihydrochloride substrate. The filter papers were moistened with sterile distilled water. The isolates were collected with a wooden stick and smeared on the filter paper.

Results

Results shows that *Bacillus* spp were isolated as shown in Table (7). Most of the *Bacillus* spp belong to *Bacillus licheniformis* and *Bacillus subtilis*. In the same vein, Romo-Barrera et al, 2021 stated that the induction of METS by bacteria of the *Bacillus* genus is a mechanism that participates in controlling the probiotic and potentially pathogenic bacteria such as *S. aureus*. The induction of METs to control pathogens may be a novel mechanism that could explain the beneficial effects of probiotics of the genus *Bacillus*.

The degradation profiles of the *Bacillus* species are shown in Table 1-6 for all the pesticide from Dat 1-21. In all, Lara force presented the best of the degradation from Day 1 to 21(0.3361.848) while Ridoff presented low degradation rate from Day 1 to 21(0.600-1.731). Rousseau et al., 2026 also stated in their research that the Rapid degradation of Lambda-Cyhalothrin makes treated vegetables relatively safe for consumption

The degradation index of the organism was calculated by dividing the number of days over the organisms counted in the culture plate at the end of 24hours, and the results are shown in Table 1(b)-6(b).

Table (1)a Degradation profiles of pesticides for Day 1

Pesticides (Tradename)	Quantity of pesticides in media	A	B	C	Average
Ridoff	1ml	0.612	0.592	0.597	0.600
Ridoff	5ml	0.641	0.656	0.638	0.645
Lara force	1ml	0.243	0.255	0.244	0.247
Lara force	5ml	0.335	0.332	0.340	0.336

Table (1)b

Colony Count result for Day 1

Pesticides (Tradename)	Quantity pesticides media	of lution used in	Number of organisms counted	Colony forming unit(cfu/ml)	radation Index
Ridoff	1ml	10 ⁻³	195	1.95x10 ⁵	0.0051
Ridoff	1ml	10 ⁻⁴	182	1.82x10 ⁶	0.0055
Ridoff	1ml	10 ⁻⁵	178	1.78x10 ⁷	0.0056
Ridoff	5ml	10 ⁻³	197	9.85x10 ⁵	0.0051
Ridoff	5ml	10 ⁻⁴	182	9.1x10 ⁶	0.0055
Ridoff	5ml	10 ⁻⁵	178	8.9x10 ⁷	0.0056
Lara force	1ml	10 ⁻³	154	1.54x10 ⁵	0.0065
Lara force	1ml	10 ⁻⁴	148	1.48x10 ⁶	0.0068
Lara force	1ml	10 ⁻⁵	127	1.27x10 ⁷	0.0079
Lara force	5ml	10 ⁻³	160	8.0x10 ⁵	0.0063
Lara force	5ml	10 ⁻⁴	153	7.65x10 ⁶	0.0065
Lara force	5ml	10 ⁻⁵	148	7.4x10 ⁷	0.0068

Table (2)a Degradation profiles of pesticides for Day 4

Pesticides (Tradename)	Quantity of pesticides in media	A	B	C	Average
Ridoff	1ml	1.330	1.352	1.325	1.336
Ridoff	5ml	1.269	1.290	1.269	1.276
Lara force	1ml	1.277	1.411	1.278	1.322
Lara force	5ml	1.427	1.412	1.433	1.424

Table (2)b Colony Count result for Day 4

Pesticides (Tradename)	Quantity of pesticides in media	oflution used in	Number of organisms counted	of Colony forming unit(cfu/ml)	radation Index
Ridoff	1ml	10^{-3}	201	2.01×10^5	0.020
Ridoff	1ml	10^{-4}	195	1.95×10^6	0.021
Ridoff	1ml	10^{-5}	184	1.84×10^7	0.022
Ridoff	5ml	10^{-3}	202	2.02×10^5	0.020
Ridoff	5ml	10^{-4}	193	1.93×10^6	0.021
Ridoff	5ml	10^{-5}	182	1.82×10^7	0.022
Lara force	1ml	10^{-3}	200	2.0×10^5	0.020
Lara force	1ml	10^{-4}	192	1.92×10^6	0.021
Lara force	1ml	10^{-5}	180	1.80×10^7	0.022
Lara force	5ml	10^{-3}	210	1.05×10^6	0.019
Lara force	5ml	10^{-4}	202	1.01×10^7	0.020
Lara force	5ml	10^{-5}	190	9.5×10^7	0.021

Table (3)a Degradation profiles of pesticides for Day 5

Pesticides Quantity of (Tradename) pesticides in media		A	B	C	Average
Ridoff	1ml	1.497	1.401	1.409	1.436
Ridoff	5ml	1.431	1.484	1.429	1.448
Lara force	1ml	1.369	1.491	1.369	1.410
Lara force	5ml	1.438	1.484	1.428	1.450

Table (3)b Colony Count result for Day 5

Pesticides (Tradename)	Quantity pesticides media	of lution used in	Number organisms counted	of Colony forming unit(cfu/ml)	radation Index
Ridoff	1ml	10 ⁻³	211	2.11x10 ⁵	0.024
Ridoff	1ml	10 ⁻⁴	207	2.07x10 ⁶	0.024
Ridoff	1ml	10 ⁻⁵	196	1.96x10 ⁷	0.026
Ridoff	5ml	10 ⁻³	201	2.01x10 ⁶	0.025
Ridoff	5ml	10 ⁻⁴	200	1.0x10 ⁷	0.025
Ridoff	5ml	10 ⁻⁵	195	1.95x10 ⁷	0.026
Lara force	1ml	10 ⁻³	199	1.99x10 ⁵	0.025
Lara force	1ml	10 ⁻⁴	183	1.83x10 ⁶	0.027
Lara force	1ml	10 ⁻⁵	175	1.75x10 ⁷	0.029
Lara force	5ml	10 ⁻³	212	1.06x10 ⁶	0.024
Lara force	5ml	10 ⁻³	199	1.99x10 ⁵	0.025
Lara force	5ml	10 ⁻⁴	183	1.83x10 ⁶	0.027

Table (4)a		Degradation profiles of pesticides for Day 7			
Pesticides (Tradename)	Quantity pesticides in media	of A	B	C	Average
Ridoff	1ml	1.606	1.606	1.606	1.606
Ridoff	5ml	1.656	1.664	1.664	1.661
Lara force	1ml	1.670	1.666	1.670	1.669
Lara force	5ml	1.645	1.648	1.648	1.647

Table (4)b Colony Count result for Day 7

Pesticides (Tradename)	Quantity pesticides media	of lution used in	Number organisms counted	of Colony forming unit(cfu/ml)	radation Index
Ridoff	1ml	10 ⁻³	225	2.25x10 ⁵	0.031
Ridoff	1ml	10 ⁻⁴	214	2.14x10 ⁶	0.033
Ridoff	1ml	10 ⁻⁵	209	2.09x10 ⁷	0.033
Ridoff	5ml	10 ⁻³	238	1.19x10 ⁶	0.029
Ridoff	5ml	10 ⁻⁴	227	1.35x10 ⁷	0.031
Ridoff	5ml	10 ⁻⁵	222	1.11x10 ⁸	0.032
Lara force	1ml	10 ⁻³	240	2.4x10 ⁴	0.029
Lara force	1ml	10 ⁻⁴	232	2.32x10 ⁶	0.030
Lara force	1ml	10 ⁻⁵	218	2.18x10 ⁷	0.032
Lara force	5ml	10 ⁻³	224	1.12x10 ⁶	0.031
Lara force	5ml	10 ⁻⁴	215	1.075x10 ⁷	0.033
Lara force	5ml	10 ⁻⁵	210	1.05x10 ⁸	0.033

Table (5)a		Degradation profiles of pesticides for Day 14			
Pesticides (Tradename)	Quantity pesticides in media	of A	B	C	Average
Ridoff	1ml	1.716	1.716	1.711	1.714
Ridoff	5ml	1.735	1.730	1.730	1.731
Lara force	1ml	1.694	1.697	1.692	1.694
Lara force	5ml	1.848	1.848	1.848	1.848

Table (5)b		Colony Count of pesticides for Day 14			
Pesticides (Tradename)	Quantity pesticides media	of lution used in	Number organisms counted	of Colony forming unit(cfu/ml)	radation Index
Ridoff	1ml	10^{-3}	Spoilt	---	---
Ridoff	1ml	10^{-4}		231 2.31x10 ⁶	0.061
Ridoff	1ml	10^{-5}		225 2.25x10 ⁷	0.062
Ridoff	5ml	10^{-3}		243 1.215x10 ⁶	0.058
Ridoff	5ml	10^{-4}		238 1.19x10 ⁷	0.059
Ridoff	5ml	10^{-5}		233 1.165x10 ⁸	0.060
Lara force	1ml	10^{-3}		260 2.6x10 ⁵	0.054
Lara force	1ml	10^{-4}		256 2.56x10 ⁶	0.055
Lara force	1ml	10^{-5}		248 2.48x10 ⁷	0.056
Lara force	5ml	10^{-3}		288 1.44x10 ⁶	0.049
Lara force	5ml	10^{-4}		284 1.42x10 ⁷	0.049
Lara force	5ml	10^{-5}		279 1.395x10 ⁸	0.050

Table (6)a Degradation profiles of pesticides for Day 21

Pesticides (Tradename)	Quantity pesticides in media	of A	B	C	Average
Ridoff	1ml	1.688	1.688	1.688	1.688
Ridoff	5ml	1.686	1.686	1.686	1.686
Lara force	1ml	1.612	1.616	1.616	1.614
Lara force	5ml		1.567	1.567	1.564 1.566

Table (6)b Colony Count of pesticides for Day 21

Pesticides (Tradename)	Quantity pesticides media	of lution used in	Number organisms counted	of Colony forming unit(cfu/ml)	radation Index
Ridoff	1ml	10 ⁻³	251	2.51x10 ⁵	0.084
Ridoff	1ml	10 ⁻⁴	230	2.3x10 ⁶	0.091
Ridoff	1ml	10 ⁻⁵	219	2.19x10 ⁷	0.096
Ridoff	5ml	10 ⁻³	Cloudy	-----	-----
Ridoff	5ml	10 ⁻⁴	228	1.14x10 ⁷	0.092
Ridoff	5ml	10 ⁻⁵	214	1.07x10 ⁸	0.098
Lara force	1ml	10 ⁻³	240	2.4x10 ⁵	0.086
Lara force	1ml	10 ⁻⁴	231	2.31x10 ⁶	0.091
Lara force	1ml	10 ⁻⁵	218	2.18x10 ⁷	0.096
Lara force	5ml	10 ⁻³	Cloudy	-----	-----
Lara force	5ml	10 ⁻⁴	231	1.155x10 ⁷	0.091
Lara force	5ml	10 ⁻⁵	210	1.05x10 ⁸	0.100

Conclusion

This investigation has clearly demonstrated that *Bacillus* species from pesticide contaminated soil have the capability to degrade Organophosphates and Pyrethroids.

Further, this demonstrates that the methods used in the isolation and identification of the bacteria confirmed that it was predominantly *Bacillus subtilis* and *Bacillus licheniformis* which had the role in degrading pesticides. The data collected clearly shows an increase in pesticide degradation with respect to time; indicating active microbial activity, metabolism and the adaptation of the micro-organism to pesticide exposure. It is apparent that the chemical structure of pesticides can influence its degradation rate by micro-organisms (as indicated by the greater rate of degradation observed in Lara force than in Ridoff). Temperature and incubation duration also appeared to significantly enhance the efficiency of degradation. Furthermore, the observed increase in Colony Forming Units (CFU) at each sampling point indicates that micro-organisms are utilizing pesticides as a carbon and nutrient source. Therefore, overall, the study confirms that *Bacillus* species have considerable Bioremediation capabilities and may therefore be utilized as effective tools in Detoxifying Pesticide contaminated Soils. Finally, using such micro-organisms would provide a sustainable, environmentally friendly and economically viable method for removing pollutants from the environment thereby providing protection for Public Health.

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