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Assessment of Biodegradation of Lambda-Cyhalothrin by Bacteria Isolated from Agricultural Soil in Elemere, Kwara State, Nigeria

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Abstract

Lambda-cyhalothrin (LC) is a synthetic pyrethroid (SP) used to control a variety of insect pests. Despite being considered potentially hazardous, LC is used in horticulture, forestry, and agriculture to control a wide variety of insect pests. AN Alternative strategy for reducing pesticide residues is biodegradation, which is both economical and environmentally benign. In this study, LC-degrading bacteria were isolated using an enrichment approach on mineral salt media. Their morphology and certain biochemical characteristics were used to characterize the isolates. For 25 days, the screened isolates' performance was monitored at various intervals using optical density. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to identify the LC degraded metabolites. Finally, the screened bacterium was identified using 16S rRNA gene sequencing. The results from the optical density showed an increase in the growth of the isolate from the 5th day to the 15th day, after which it gradually decreased until the 25th day. The GC-MS results revealed the presence of 3-phenoxyphenyl acetonitrile, cyclopropane-1-carboxylic acid, N-(Hydroxymethyl)-acetamide, phenol, phenoxy-benzoate, 3-phenoxy-acetic acid, and phosphonoacetic acid metabolites. The bacterial isolate with the best potential for LC degradation was identified as Klebsiella pneumoniae strain ABS1 and can be explored for bioremediation of LC-contaminated agricultural soils and other synthetic pyrethroids.

Keywords: Agricultural soil, Biodegradation, Gas Chromatography-Mass Spectrometry, Lambda-cyhalothrin

INTRODUCTION

Chemical pollution in the environment has detrimental effects on both human and animal health. Although chemicals were used much earlier to manage weeds, fungi, and insects, the widespread usage of pesticides did not occur until the 20th century (Gammon *et al.*, 2019). Synthetic pyrethroids (SPs) are currently the most widely used insecticides globally (Casida and Durkin, 2017). Allethrin was the first SP pesticide created in 1949, and SPs are chemical analogs of natural pyrethrins derived from Chrysanthemum cinerariaefolium flowers (Ensley, 2018; Gammon *et al.*, 2019; Xu *et al.*, 2019). Fish and wildlife mortality has increased as a result of these compounds, despite their purported lower persistence compared to organochlorines and their lack of accumulation. According to the WHO, they are classified as insecticides of the fourth group (WHO, 2016).

Lambda-cyhalothrin (LC) is a commonly used SP in agricultural settings. As a broad-spectrum insecticide, lambda-cyhalothrin [(RS)-a-Cyano-3-phenoxybenzyl-(Z) - (1RS,3RS) (2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate] is one of the primary SPs used to control a variety of insect pests in a range of domestic and agricultural applications (He *et al.*, 2018). Lambda Cyhalothrin is a cyano group-containing type II SP that induces salivation and choreoathetosis. Variations in the toxicities of the same molecule may be caused by the formation of distinct SP with different isomeric ratios (Ghosh and Rokade, 2012). Both ecosystems and human health are seriously threatened by the excessive and careless use of LC (Lubick, 2008; Alonso *et al.*, 2012). Numerous studies have shown that high doses of exposure can have serious negative health effects, including neurotoxicity, in mammals (Soderlund, 2020). Additionally, even at low levels, long-term exposure to LC may raise the risk of infantile leukemia (Ding, 2012), mutagenicity and carcinogenicity (Saleem, 2014). Furthermore, fish and aquatic invertebrates are extremely poisoned by lambda-cyhalothrin (Kumar *et al.*, 2012). Numerous indicators suggest that LC poses a threat to ecosystems and human health (Alonso *et al.*, 2012).

Biodegradation has emerged as an eco-friendly and cost-effective approach for the removal of lambda-cyhalothrin residues from contaminated environments. Microbial degradation not only reduces the concentration of the parent compound but also minimizes the formation of toxic intermediates, making it a promising strategy for mitigating the ecological and health risks associated with LC contamination (Zhang *et al.*, 2023). Effective methods to eliminate LC from the environment are urgently needed, as bacteria have been shown to have considerable potential for biodegrading several organic chemicals, including pesticides (Tamil and Syed, 2018; Abdulsalam *et al.*, 2023a).

MATERIALS AND METHODS

Chemicals

The technical grade lambda-cyhalothrin, Karate 5EC (emulsifiable concentrate), was purchased from an agrochemical retailer in Malete Market, Kwara State, Nigeria. All other chemicals used in this study were of analytical grade and were purchased from the Central Research and Diagnostic Laboratory, Ilorin, Kwara State, Nigeria.

Soil Sample Collection and Preparation

The study involved a farm in Elemere town (8.6856917°N, 4.514993°E) in the Moro Local Government Area, Kwara State, Nigeria, that had a history of repeated Lambda-cyhalothrin insecticide use. Aseptic technique was employed to collect soil samples for this study in December 2021. For the sampling, ten randomly selected locations were used. Each farming section had one kilogram of soil removed from a depth of 0 to 15 cm, which was then combined into a single sample. According to Moghaddam *et al.* (2011), the composite sample was brought to the lab in sterile plastic bags. The collected soil samples were also hand-picked to eliminate plant debris and fauna, and the soils were then gently sieved (Estefan *et al.*, 2013; Wang *et al.*, 2017).

Isolation of Bacterial Isolates

Bacteria were isolated using enrichment procedures in mineral salt medium (MSM) (Zhang *et al.*, 2023). As the only source of carbon and energy, five grams of the pesticide-contaminated soil sample was added to 100 milliliters of MSM medium supplemented with 20 milligrams per liter of lambda-cyhalothrin (LC). The mixture was then shaken at 160 rpm at 37 degrees Celsius for seven days. The culture medium was moved into a new MSM enriched with 20 mg/L of LC after seven days.

For an additional seven days, the medium was incubated on a shaker at 160 rpm and 37 °C. To allow LC-degrading bacteria to proliferate, this process was repeated four times with progressively higher LC concentrations, reaching a maximum of 100 mg/L. Until pure cultures were achieved, the bacterial isolates were cultivated on nutrient agar multiple times (Parte *et al.*, 2017).

Characterization of the LC-Degrading Bacterial Isolate

Colonial characteristics of five distinct LCDB-degrading isolates were observed after growing on a nutrient agar plate for 24 hours at 37 °C. Gram staining and biochemical tests were also performed as described by Shahzad et al. (2012) and Arora and Bae (2014).

Biodegradation of Lambda-Cyhalothrin by LCDB7 Isolate

Out of the five bacteria identified during the enrichment, the isolate LCDB7 was selected because it was the best degrader among the five isolates. A flask of mineral salt medium containing 100 mg/L of LC was inoculated with an aliquot of the standardized suspension of the LCDB7 isolate. Growth was tracked using optical density (turbidity) measured at intervals of 1, 5, 10, 15, 20, and 25 days using a spectrophotometer (UV/Vis 721 (D): Axiom Medical Ltd., UK) at 620 nm. The flask was incubated for 25 days at 37 °C on a rotating shaker running at 160 rpm (Parte *et al.*, 2017). The control was an uninoculated flask containing 100 mg/L of LC without the isolate in a mineral medium.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To obtain a cell-free medium, 10-milliliter aliquots were extracted from both the treatment and control samples at 5-day intervals. The aliquot was then centrifuged for 10 minutes at 8000 rpm. In a separating funnel, an equivalent volume of ethyl acetate was introduced to the corresponding cell-free media and let to settle. Following separate collection, the organic layer, consisting of ethyl acetate, was aspirated, pooled, and allowed to evaporate at room temperature. A gas chromatography (Hewlett Packard 984-BMS) system, fitted with a Resteck column (0.25 mm × 30 mm; XTI-5), connected to a mass spectrometry, was then used to evaluate the centrifuged samples and the control. The temperature programming mode was changed, and samples were injected in splitless mode. Helium was used as the carrier gas, and the compounds were identified based on mass spectra and compared using the National Institute of Standards and Technology (NIST) library (NIST, 2018). The initial temperature of the column was maintained at 800 °C for two minutes, then increased by 100 °C, and finally reached a final temperature of 290 °C, where it was held for five minutes.

Molecular Identification of the LC-Degrading Bacterial Isolate

Using 16S rRNA gene sequencing homology, isolate LCDB7 was further identified. Utilizing the Presto™ Mini g DNA bacterium kit from Geneaid Biotech Ltd. (Taiwan), the isolate's DNA was extracted (Zhang *et al.*, 2023). The universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), totaling 50 µL, were used to amplify the isolated DNA using the polymerase chain reaction (PCR) method. For three minutes, the first denaturation process was carried out at 96 °C. Twenty-seven cycles of 96 °C for 30 seconds came next. After that, annealing was carried out for 25 seconds at 56 °C, followed by extension for 15 seconds at 72 °C, and finally, extension was performed for 10 minutes at 72 °C (Swaathy *et al.*, 2014). A 1% (w/v) agarose gel prepared with TBE buffer was used to separate the PCR products according to their molecular weight. An ethidium bromide DNA stain was used to visualize the DNA under ultraviolet light. Following that, the amplified DNA underwent purification and sequencing. Inqaba Biotec in South Africa is where the nucleotide sequences were ascertained. To evaluate the sequence homology and identify the isolates, the generated 16S rRNA gene sequences were corrected and compared with NCBI nucleotide sequences using BLAST tools. CLUSTAL Omega was used to perform the multiple sequence alignment ("https://www.ebi.ac.uk/Tools/msa/clustalo/"). The nucleotide sequences were submitted to the NCBI GenBank database for accession number assignment, and the phylogenetic tree was constructed using MEGA version 7.0 (Swaathy *et al.*, 2014; Abdulsalam *et al.*, 2023b).

RESULTS

Morphological and Biochemical Characteristics

All five bacterial isolates were tentatively identified as *Bacillus* species I and II, *Klebsiella* species, *Pseudomonas* species, and *Lysinibacillus* species (LCDB5-LCDB9, respectively) based on morphology and biochemical tests (Table 1).

Table 1: Morphological and Biochemical Characteristics

Characteristics	LCDB5	LCDB6	LCDB7	LCDB8	LCDB9
Gram reaction	+	+	-	-	+
Cell Form	Rod	Rod	Rod	Rod	Rod
Spore Formation	+	+	-	-	+
Motility	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	+	-	+	+
Urease	+	+	+	+	-
Citrate Utilization	-	-	+	+	-
Indole	-	-	-	+	-
Methyl Red	-	-	-	+	-
Voges Proskaeur	-	-	+	-	+
Hydrogen Sulphide Production	-	-	-	-	-
Starch Hydrolysis	+	+	+	-	-
Glucose Fermentation	+	+	+	+	-
Lactose Fermentation	-	-	+	+	-
Sucrose Fermentation	+	-	+	+	-
Probable Isolates	*Bacillus* species I	*Bacillus* species II	*Klebsiella* species	*Pseudomonas* species	*Lysinibacillus* species

KEYS: +: Positive Reaction; -: Negative Reaction

Biodegradation of Lambda-Cyhalothrin by LCDB7 Isolate

The growth rate (turbidity) of the isolate increased from the fifth day to the fifteenth and then gradually decreased from the sixteenth day to the twenty-fifth day, while the control showed no change in growth from day one to day twenty-five (Table 2).

Table 2: Optical Density Showing the Growth Rate of LCDB7 (620nm)

Isolate /Days	1	5	10	15	20	25
LCDB7	0.077	0.323	0.795	1.356	1.110	1.015
Control	0.002	0.003	0.001	0.002	0.002	0.002

Identification of Metabolites produced by the isolate

The metabolites produced after biodegradation with the isolate were identified using Gas Chromatography-Mass Spectrometry (GC-MS). At m/z 449.90, a prominent peak was seen with a distinctive mass fragment that is indicative of the LC parental ion. The metabolite's retention duration was 27.729 minutes, matching the genuine standard found in the database of the National Institute of Standards and Technology (NIST, USA). During the biodegradation process, the parent chemical vanished, resulting in the formation of seven new metabolites, which were identified as 3-phenoxyphenyl acetonitrile, Cyclopropane-1-carboxylic acid, N-(Hydroxymethyl)-acetamide, Phenol, Phenoxy-benzoate, 3-Phenoxy-acetic acid, and Phosphonoacetic acid were chosen because they resembled the real compounds in the NIST library database in terms of molecular ions and retention duration (Table 3). For the first time, phosphonoacetic acid was identified in the degradation pathways of SPs among the metabolites discovered. The metabolites found led to the proposal of an LC degradation route by LCDB7 isolate (Figure 1). LC was first broken down by hydrolysis, which caused the ester link to cleave. The breakdown of the aromatic ring, followed by the cleavage of the diaryl bond, further altered LC. Ultimately, LCDB7 broke down LC without producing any harmful byproducts.

Table 3: Lambda-Cyhalothrin Metabolites After Biodegradation with LCDB7 Isolate

Metabolites	Retention Time (RT/mins)	m/z	Chemical Name
M1	27.729	449.90	Lamda-Cyhalothrin
M2	23.009	209.24	3-Phenoxyphenyl acetonitrile
M3	4.495	86.10	Cyclopropane-1-carboxylic acid
M4	7.968	89.09	N-(Hydroxymethyl)-acetamide
M5	10.497	140.03	Phosphonoacetic acid
M6	13.943	152.15	3-Phenoxy-acetic acid
M7	18.762	198.22	Phenoxy-benzoate
M8	9.988	94.11	Phenol

The Phylogenetic Analysis of the LCDB7 Isolate

The results obtained from the analysis of the 16S rRNA confirmed that the strain LCDB7 is *a Klebsiella pneumoniae strain, specifically* ABS1. The phylogenetic tree is shown in Figure 2.

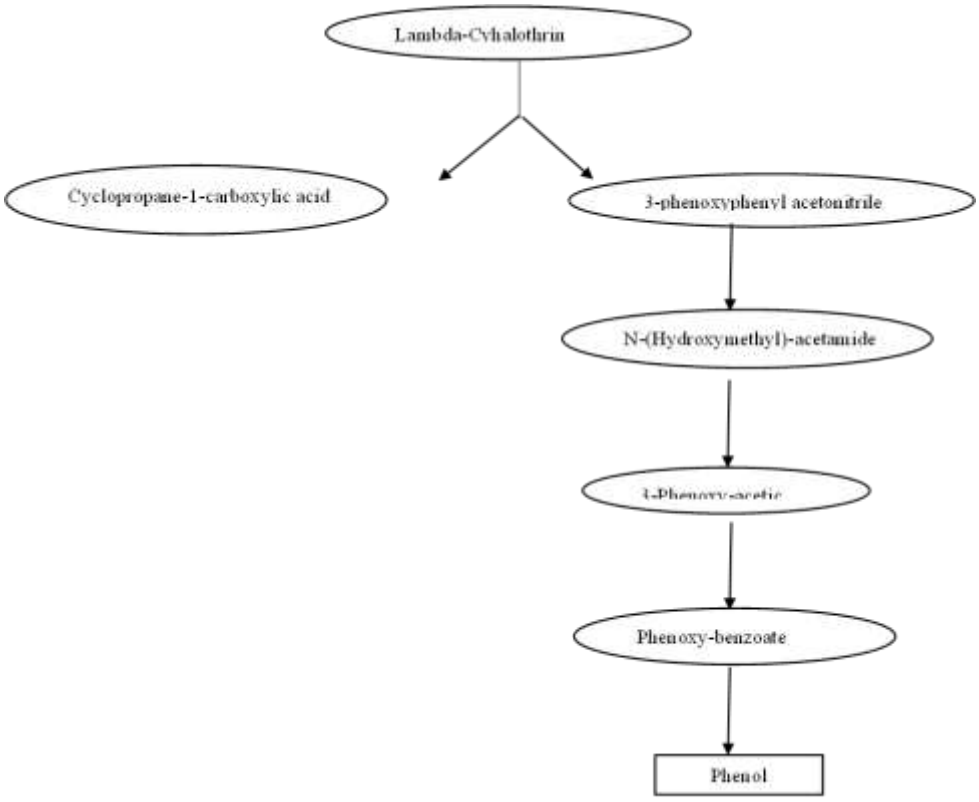

Figure 1: Proposed Lamba-Cyhalothrin Biodegradation Pathway in *Klebsiella pneumoniae* strain ABS1

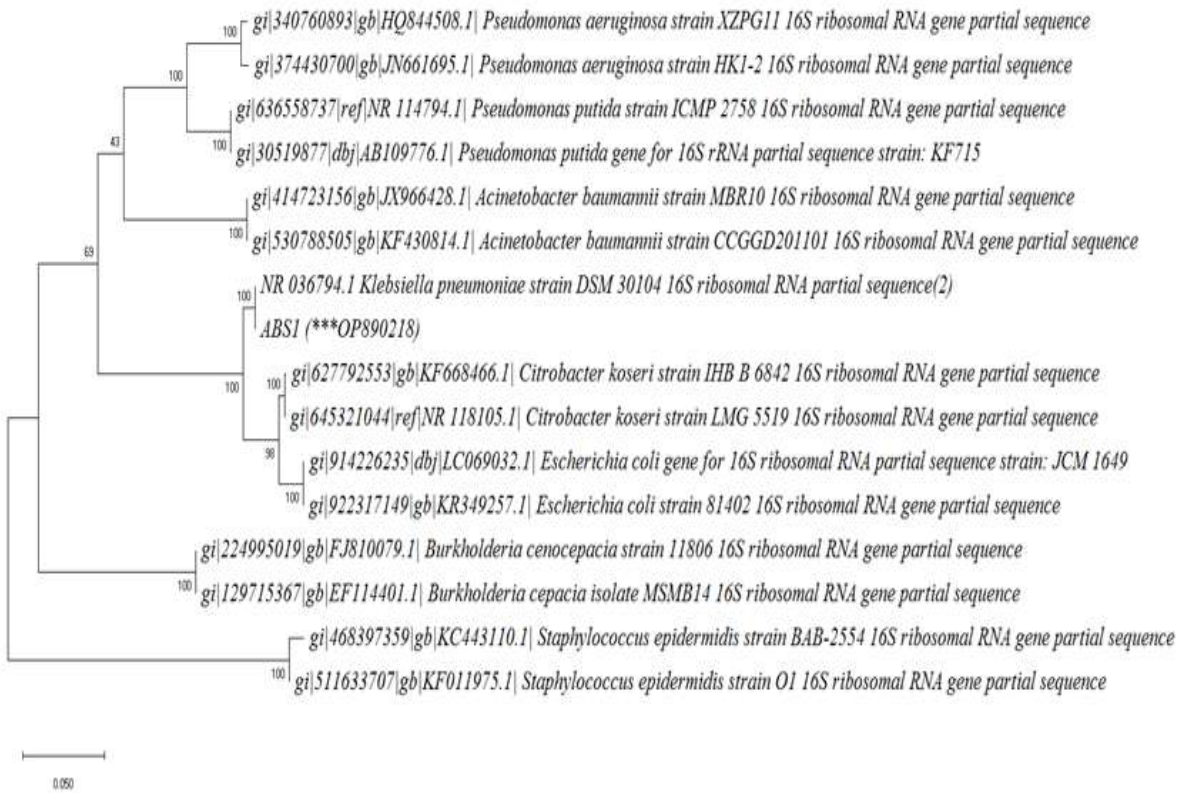

Figure 2: The Phylogenetic Tree of *Klebsiella pneumoniae* strain ABS1

DISCUSSION

Based on morphological and biochemical characterization, the LC-degrading isolates were identified as Lysinibacillus sp. (LCDB9), Pseudomonas sp. (LCDB8), *Klebsiella sp.* (LCDB7), and two Bacillus species (LCDB5 and LCDB6). The results of Chen *et al.* (2015), who isolated a strain of *Bacillus thuringiensis* from contaminated agricultural soil, are comparable to this result. This study's findings align with those of Cycon and Piotrowska-Seget (2016), who demonstrated that both fungi and bacteria are capable of degrading pyrethroids in liquid cultures and soils. Similarly, Anjos *et al.* (2020) reported that Lysinibacillus xylanilyticus can break down synthetic pyrethroids. The results also corroborate the work of Abdulsalam *et al.* (2023a), who identified four distinct Bacillus strains with strong potential for LC degradation, as well as Uzma et al. (2023), who isolated various Pseudomonas species from soils contaminated with LC. Collectively, these findings highlight the rich diversity of microorganisms present in contaminated soils.

The optical density measurements indicated an increase in the population of isolate LCDB7 up to the fifteenth day. This observation is in agreement with the findings of Manigandan and Nelson (2015), who reported a similar rise in optical density for *Stenotrophomonas maltophilia*. It also supports the results of Monica *et al.* (2016), who observed increased optical densities up to the twelfth day when using a consortium of microorganisms. Furthermore, these results are consistent with those of Abdulsalam *et al.* (2023a), who documented a rise in optical densities for four *Bacillus* species during LC degradation up to day six.

The GC-MS analysis revealed distinct peaks at various retention times, indicating the formation of different compounds during the degradation of LC. This observation is consistent with Saied et al. (2021), who also reported significant chemical peaks resulting from the biodegradation of cypermethrin by *Lysinibacillus cresolivuorans* HIS7. Similarly, Chen and Zhan (2019) noted that microorganisms can degrade pyrethroids either through co-metabolization or by directly utilizing them as a carbon source. These findings are further supported by Bhatt et al. (2020), who observed varying retention times and peak patterns following the biodegradation of cypermethrin by *Bacillus thuringiensis* strain SG4. Overall, the results of this study demonstrate the production of diverse metabolites during LC breakdown, in line with previous research.

The identification of intermediate molecules confirmed the metabolite analysis results, indicating that carboxylesterases were primarily responsible for hydrolyzing the LC ester bond and generating acid and alcohol derivatives. This finding aligns with the work of Bhatt *et al.* (2020) and Xu *et al.* (2020), who demonstrated that ester bond hydrolysis is the initial step in the biodegradation of synthetic pyrethroids. Similarly, Zhan *et al.* (2020) reported that various bacterial species are capable of hydrolyzing the ester linkage and degrading synthetic pyrethroids. The detection of phosphonoacetic acid in this study is also consistent with the observations of Chen *et al.* (2015) and Birolli et al. (2019), who documented the formation of different acids during biodegradation experiments with *B. thuringiensis* and a consortium of four *Bacillus* species, respectively.

Following the 25-day incubation period, *Klebsiella pneumoniae* strain ABS1 activity caused the breakdown of macromolecular components into smaller ones. The metabolic routes of many pyrethroid insecticides were revealed by Zhao *et al.* (2020) and others. Of these, 3-phenoxybenzoic acid (3PBA) is the most lethal SP intermediate. In this investigation, 3PBA was surprisingly absent, suggesting that the isolate could break down LC without producing harmful metabolites. This demonstrates that strain LCDB7 was capable of breaking down LC into simple, non-toxic chemicals, as all toxic intermediates vanished after extended incubation.

The phylogenetic analysis of the LCDB7 isolate identified it as a *Klebsiella pneumoniae* strain, ABS1, with an accession number of OP890218. These outcomes align with Tang et al.'s (2019) findings, which demonstrated the ability of *Klebsiella pneumoniae* BPBA052 to degrade SPs and their metabolites. According to Tang *et al.* (2020), deltamethrin was also broken down by a co-culture of K*lebsiella pneumoniae* BPBA052 and *Acinetobacter junii* LH-1-1. Onuorah *et al.* (2021) state that *Pseudomonas putida*, *Lysinibacillus macrolides*, and *Klebsiella pneumoniae* can break down lambda-cyhalothrin and dichlorvos.

CONCLUSION

Klebsiella pneumoniae strain ABS1 demonstrated significant biodegradation competence for Lambda-cyhalothrin (LC), as confirmed by the absence of toxic intermediates in its metabolic byproducts. This study elucidates the bacterial degradation pathway for LC, indicating that ABS1 achieves complete and efficient mineralization of the compound. Consequently, this strain holds promise for large-scale application in bioremediation strategies targeting environments contaminated with LCs.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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