

ISOLATION CHARACTERIZATION AND DIVERSITY OF INDIGENOUS PESTICIDE DEGRADING MICROBES FROM SELECTED AGRO ECOLOGICAL ZONES OF MALAWI

ABSTRACT

Pesticide xenobiotics have a great impact on bio argumentation, bio-magnification, and environment degradation regardless of being adopted by green revolution technologies. Bioremediation is widely accepted because it's cheap, practical at the same time environmentally friendly. Bioremediation advocates indigenous microbes use to degrade pesticides, therefore a study has been performed to show prospects of degrading microorganisms. The study isolated microbes from different agro ecological zones to assess their capacity to utilize some pesticide as sole carbon source complimented by the presence of laccase gene. Biochemical test and genetic characterization using 16S rDNA genes were used in identification. Diversified species and strains of genus *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Pantoea* and *Leclercia*, were found to degrade cypermethrin and acetochlor but no microbe was found to degrade dimethoate. The study adds new strain of microbes involved in degradation of cypermethrin and acetochlor and also strains that that can degrade both. The study puts proposition that pest infestation in fields is a result of abundance of xenobiotic degrading microbes due to natural selection pressure not pesticide resistance of the pest.

Keywords: Pesticide, Cypermethrin, Dimethoate, Acetochlor, indigenous soil microorganisms, Bioremediation, Xenobiotics

1. INTRODUCTION

Pest infestation is one of the causes of decrease in yield and yield components in Malawi. Green revolution techniques prefer the usage of pesticides backed by advantages of being quick, more effective, time and labor saving than , cultural, mechanical and biological methods 1,2. Currently, the use of different pesticides (herbicides and insecticides) in one field is essential to control diverse weeds and insects due to the compatibility and effectiveness of pesticides 3.

Pesticides which include herbicides and insecticides are of paramount importance for controlling weeds and insects respectively by farmers in Malawi 4. However, regardless of benefits, pesticides contain xenobiotics which have a negative impact on bio-magnification, beneficial soil microbes, 5,6 and ecosystem 7. They have a direct and indirect impact to yield components and yield because they interfere with soil-beneficial organisms and plant nutrition 8, 9.

There are several clean-up mechanisms for pesticides xenobiotics; volatilization, chemical treatment methods, and incineration. These methods have not been accepted by public due to association with large volumes of acids and alkalis which create a problem of disposal and emissions of toxic chemicals 10, 11. These methods are also inefficient, not practical and expensive because the polluted soils can't be extracted, transported, treated, and replaced 12.

It's for these reasons that biological methods involved in biodegradation are preferred 11 13 14. This involves the use of microorganisms to degrade xenobiotics by a process called bioremediation 11. Its advantageous because some microbes involved in bioremediation can fix nitrogen fixation, produce phytohormones and solubilize phosphorus 15. Bioaugmentation by biostimulation of indigenous microbes is promising technique when dealing with heavily and/or historically contaminated sites.

Previous studies have documented that microbes play important roles in the detoxification and degradation of xenobiotics 16–20. Thus far, many reports have described the biodegradation of

cypermethrin and acetochlor by various microorganisms but there is no research describing isolation and characterization of indigenous microbes involved in degradation of these pesticides in Malawi and its neighboring countries except for glyphosate 21.

The research aim was to identify the potential microbial strain with ability to degrade cypermethrin, dimethoate and acetochlor from the contaminated soil of Malawi. In this study, the isolation and characterization of cypermethrin, dimethoate and acetochlor degrading microbes is examined with the hope of creating an environment free from xenobiotics which have an effect in contamination of farms, and aquatic environments.

2. METHODOLOGY

2.1. Soil Samples Collection

The procedure was as described by Eman et al., (2013) with some modifications whereby Soil samples were collected from sites having a long history of pesticides application except Champhoyo farm which had 1-year history of pesticide application. Soil samples were collected from the 3-15 cm top layer of cultivated soil from several plots of the farms. The sites were Champhoyo farm in Karonga district, Nkhozho farm (Exagris estates) in Rumphi district and Tea Estate in Mulanje district. Samples were collected in polythene bags and placed in iced cooler boxes during transportation to the laboratory and stored at 4 °C. 3 places were identified for sampling from each sampling site namely; 500m outside the area of farm where no history of application of the pesticide (upstream of drainage and wind), inside the farm with long history of pesticide, and downstream the drainage system of the farm.

2.2. Types of Pesticides Used

The pesticides used in this study were cypermethrin, Dimethoate and acetochlor which were purchased from the Farmers Organisation Limited shop.

2.3. Isolation of Microorganisms

Microorganisms were isolated from soil samples using the enrichment culture technique. Firstly 5 gm of soil sample was put into a 250 ml flask containing 50 ml of sterile liquid Mineral Salt Medium (MSM) with 100 ppm of pesticide was used for isolating degrading microbes. Mineral Salt Medium (MSM) containing (g/l) KH_2PO_4 (1.5), Na_2HPO_4 (0.6), NaCl (0.5), NH_4SO_4 (2), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2), CaCl_2 (0.01), and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.001) was used. Microbe isolation was carried out at different concentrations of pesticides (100, 200, 1000 and 10000 ppm) on Czapek Dox agar 22,23. Microbes that tolerated pesticide up to 1000 ppm were considered for further studies

2.4. Determination of Pesticide Utilization Patterns

These were modifications of Shamsuddeen & Inuwa, (2013) and Akbar et al., (2015) where individual microorganisms that were tolerant to pesticides were transferred into three 250-ml flask which had 50 ml MSM 1, MSM 2 and MSM 3 with the addition of 20 mls of pesticides as sole carbon, phosphorous sources or utilization both for 40 days 28 °C. Negative Controls were not inoculated. The composition of MSM-1 was KH_2PO_4 (1.5), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2), Na_2HPO_4 (0.6), NaCl (0.5), NH_4SO_4 (2), CaCl_2 (0.01) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.001) in grams dissolved in 1 L of water, pH (7.0). MSM-2 had no phosphate source and targeted pesticides to be sole P source, and had the following composition: glucose (10), Tris buffer (12), CaCl_2 (0.01), NaCl (0.5), NH_4SO_4 CaCl_2 (0.01) and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.001) in grams dissolved in 1 liter of distilled water, pH (7.0). MSM-3 was used for isolating microbes using pesticides as the sole P and carbon source with the following composition: NaCl (0.5), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2), KCl (0.5) NH_4SO_4 (2). Growth of microorganisms which is an indicator of degradation was observed by inoculating 1 ml of individual isolate in MSM after 40 days on Czapek Dox agar plates at 28 °C. Second growth was quantified by checking isolates periodically by measurement of the turbidity at 625 nm using a spectrophotometer but for fungus development of spores or hyphae on top of media.

2.5. Determination of Laccase Enzyme

To determine the presence of laccase enzyme production isolates were streaked on Sabouraud Dextrose Agar (SDA), amended with 1% ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) by streak method. Positive test for production of laccase enzyme was confirmed by the development of dark green to purple colour around the colonies 24.

2.6. Determination of Plant Growth Regulatory Traits

To determine if isolates had Plant Growth Regulatory Traits (PGRT) the following biochemical tests were conducted:

2.6.1. Determination of Ammonia Production

Broth cultures of isolated microbes were inoculated in 10 mL tube of peptone water and incubated at $36 \pm 2^\circ\text{C}$ for 48-72 hrs. There after 0.5 mL of Nessler's reagent was added. Positive test for production of ammonia was confirmed by the development of yellow or brown colour 8.

2.6.2. Determination of Indole Acetic Acid Production

The production of IAA by isolated microbes was determined as described by Ahmad et al., (2008) with slight modification. The isolates were grown in NB amended with tryptophan (100 $\mu\text{g/ml}$) set at 30°C for 48 hours in an orbital incubator while shaking at 120 rpm. Broth media with isolates was centrifuged at 3000 rpm for 30 minutes. Thereafter 2 ml supernatant was recovered and 2 drops of o-phosphoric acid and 4 mls of Salkowski reagent were added in succession. A Positive test for production of IAA was confirmed by development of pink.

2.6.3. Determination of Catalase Production

This was done by adding 2 drops of 3% hydrogen peroxide to grown culture of isolated microbes on a slide using wire loop in a biosafety cabinet. Positive test for production of catalase was confirmed by effervescence 25–30.

2.6.4. Determination Hydrogen Cyanide Production

Isolated microorganisms were tested in-vitro for Hydrogen Cyanide (HCN) production by the method described by Ahmad et al., (2008) with some modifications. Isolates grown in nutrient broth and Sabouraud Dextrose broth amended with glycine 4.4 g/L were inoculated on modified NA and SDA plates for bacteria and fungi respectively. Sterile Whatman filter paper No. 1 was dipped in 2.5% sodium carbonate in 0.5% picric acid solution and later placed on top of the grown cultures on the agar plate. Agar Plates were tightly sealed and incubated for four days. A Positive test for production of HCN was confirmed by colour change from yellow to orange-red colour on Whatman filter paper.

2.6.5. Determination for The Production of Siderophores

Qualitative Production of Siderophore by isolates was done using universal chrome azurol S (CAS) agar plate assay as documented by Liu et al., (2016). This was done using CAS agar plates, because siderophore producing microbes form an orange halo around the colonies. This is so because Fe is changed from its original blue CAS–Fe (III) complex during the production of siderophore. After 7 days' incubation (28°C) plates were observed for the production of halo zone 8.

2.6.6. Determination for Nitrogen-Fixing Ability

Nitrogen fixing ability of microbes was assessed using Liu et al. (2016) with some modifications by manipulating the pH to 6. The isolates were streaked on winogradsky's N-free medium incubated at 28°C for 7 days. The growth of isolates on the media was considered as an ability to fix atmospheric nitrogen.

2.7. Cell Morphology and Colonies

The isolates were preliminary observed for colony morphology using a magnifying glass. Later morphological characters like colony surface texture, margins, elevation, pigmentation and shape, were observed using microscope. Gram staining was used to study cell structure, shape and size.

2.8. Identification of The Microbes by Genetic Analysis

Characterizations of isolates were done by sequencing 16S rDNA gene. Genomic DNA was extracted and purified using the ZR-kit following manufacturer's manual and amplified using Polymerase Chain Reaction (PCR) of the 16S rDNA supplied by Inqaba <http://www.inqababiotec.co.za>. The primers were 907R (5'- CCGTCAATTCMTTTRAGTTT-3') and 1492R (5'- TACGGYTACCTTGTTACGACTT-3') and Sequencing of the isolates 16S rDNA was done by Inqaba Biotech Ltd in South Africa using Sanger sequencing. A consensus sequence of two PCR products of 16S rDNA sequence data was done using BioEdit software. The consensus sequence obtained in BioEdit was analyzed by BLAST algorithm for comparison of a nucleotide query sequence against public nucleotide sequence database to find the closely related strains. The nucleotide sequences of the 16S rDNA were subjected to BLAST analysis with the National Center for Biotechnology Information (NCBI) database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences with high similarity scores were downloaded from the NCBI database. Based on maximum identity score first sequence was selected and aligned with isolate sequences using multiple alignment software program MUSCLE Distance matrix was generated using RDP database. The first step the Neighbour Joining method was used for defining dataset because it establishes relationships between sequences according to their genetic distance (a phenetic criterion) alone, without taking into account an evolutionary model. Later Maximum Likelihood was used to investigate the space of all possible phylogenetic trees, trying to identify those that are best taking into consideration of all possible trees to identify the best ones. Phylogenetic tree was constructed using Seaview. All sequences were deposited in the GenBank sequence database, and the accession numbers were obtained.

3. RESULTS

All microbes that were found to grow on 100 ppm of pesticide were the same microorganism tolerating pesticide up 1000 ppm and also same microbes which utilized pesticide as sole carbon source. Isolate 3106br (2106 r) was identified to degrade both cypermethrin and acetochlor as indicated in table one. Nine strains of bacteria were found to degrade cypermethrin (*Serratia marcescens*, *Pseudomonas*, *Leclercia* sp., 2 strains of *Klebsiella pneumoniae* and 4 strains of *Enterobacter*) while 11 strains of bacteria were found to degrade acetochlor (*Pantoea agglomerans*, and 10 strains of *Enterobacter*) from 3 sites as shown in table 1 and 2. Pest infestation is one of the causes for decrease in yield and yield components in Malawi. Green revolution techniques prefer usage of pesticides backed by advantages of being quick, more effective, time and labor saving than , cultural, mechanical and biological methods^{1,2}. Currently the use of different pesticides (herbicides and insecticides) in one field is essential to control diverse weeds and insects due to compatibility and effectiveness of pesticides³.

Pesticides which include herbicides and insecticides are of paramount importance for controlling weeds and insect respectively by farmers in Malawi⁴. However, regardless of benefits,pesticide contain xenobiotics which have a negative impact on bio-magnification,beneficial soil microbes, ^{5,6} and ecosystem⁷. They have direct and indirect impact to yield components and yield because they interfere with soil beneficial organism and plant nutrition^{8,9}.

There are several clean-up mechanisms for pesticides xenobiotics; volatilization, chemical treatment methods and incineration. These methods have not been accepted by public due to association with large volumes of acids and alkalis which create a problem of disposal and emissions of toxic chemicals ^{10,11}. These methods are also inefficient, not practical and expensive because the polluted soils can't be extracted, transported, treated and replaced ¹².

It's for these reasons that biological methodsinvolved in biodegradation are preferred ^{11,13,14}. This involves the use of microorganisms to degrade xenobiotics by a process called bioremediation ¹¹. Its advantageous because some microbes involved in bioremediation can fix nitrogen fixation, produce

phytohormones and solubilizing phosphorus¹⁵. Bioaugmentation by biostimulation of indigenous microbes is promising technique when dealing with heavily and/or historically contaminated sites.

Previous studies have documented that microbes play important roles in detoxification and degradation of xenobiotics¹⁶⁻²⁰. Thus far, many reports have described the biodegradation of cypermethrin and acetochlor by various microorganisms but there is no research describing isolation and characterization of indigenous microbes involved in degradation of these pesticides in Malawi and its neighboring countries except for glyphosate²¹.

The research aim was to identify the potential microbial strain with ability to degrade cypermethrin, dimethoate and acetochlor from the contaminated soil of Malawi. In this study, isolation and characterization of cypermethrin, dimethoate and acetochlor degrading microbes is examined with the hope of creating an environment free from xenobiotics which have an effect in contamination of farms and aquatic environments.

Table 1: List and biochemical tests of pesticide degrading microbes isolated from selected agro ecological zones of Malawi

LAB NO	Microbe	Site	Pesticide	PS	IAA	Gram	Amm	Catal	Sider	Shap	sole source			Lacca
											a	P	Ca & P	
3106r	<i>Enterobacter</i>	M	C	+	+	-	+	+	+	R	+	-	-	+
3103	<i>Enterobacter</i>	N	C	+	+	-	+	+	+	R	+	-	-	+
3100 a	<i>Enterobacter cloacae</i>	N	C	+	+	-	+	+	+	R	+	-	-	+
3100 b	<i>Klebsiella pneumoniae</i>	N	C	+	+	-	+	+	+	R	+	-	-	+
3106b r	<i>Enterobacter asburiae</i>	M	C	+	+	-	+	+	+	R	+	-	-	+
3104 b	<i>Klebsiella pneumoniae</i>	K	C	+	+	-	+	+	+	R	+	-	-	+
3106b	<i>Leclercia sp.</i>	M	C	+	+	-	+	+	+	R	+	-	-	+
3102	<i>Klebsiella oxytoca</i>	N	C	+	+	-	+	+	+	R	+	-	-	+
3104 a	<i>Pseudomonas aeruginosa</i>	K	C	+	+	-	+	+	-	R	+	-	-	+
2101	<i>Enterobacter cloacae</i>	N	A	+	+	-	+	+	+	R	+	+	+	+
2106 r	<i>Enterobacter asburiae</i>	M	A	+	+	-	+	+	+	R	+	+	+	+
2100a	<i>Enterobacter asburiae</i>	N	A	+	+	-	+	+	+	R	+	+	+	+
2100B	<i>Enterobacter cancerogenus</i>	N	A	+	+	-	+	+	+	R	+	+	+	+
2103-2	<i>Enterobacter tabaci,</i>	N	A	+	+	-	+	+	+	R	+	+	+	+
2103	<i>Enterobacter asburiae</i>	N	A	+	+	-	+	+	+	R	+	+	+	+
2106a	<i>Enterobacter xiangfangensis</i>	M	A	+	+	-	+	+	+	R	+	+	+	+
2104-	<i>Enterobacter cloacae</i>	K	A	+	+	-	+	+	+	R	+	+	+	+
2106b	<i>Enterobacter xiangfangensis</i>	M	A	+	+	-	+	+	+	R	+	+	+	+
2107b	<i>Pantoea agglomerans,</i>	M	A	+	+	-	+	+	+	R	+	+	+	+
2104	<i>Enterobacter cloacae</i>	K	A	+	+	-	+	+	+	R	+	+	+	+
2105	<i>Enterobacter tabaci,</i>	M	A	+	+	-	+	+	+	R	+	+	+	+

C=cypermethrin, A=Acetochlor, B=bacteria, M=Mulanje, K=Karonga, N=Nkhozho, SI=solubilization index, Y= coccobacilli, R=Rod shaped, PS=Phosphate solubilization, Ca=carbon, P=phosphorous IAA=Indole Acetic Acid

Table 2: Distribution of pesticide degrading microbes within the application catchment area

♣	Karonga			Rumphi (Nkhozho)			Mulanje		
	Outsid	Inside	Downstream	Outsid	Inside	Downstrea	Outside	Inside	Downstrea

	e the farm	the farm	(drainage system)	e the farm	the farm	m (drainage system)	the farm	the farm	m (drainage system)
C		3104b 3104a	3104 b		3103 3100a 3100b 3102	3100b 3102	3106 3106r 3106b	3106 3106r 3106b	3106 3106r 3106b
A		2104	2104		2101 2100a 2100B 2103-2 2103	2103-2 2103	2106r 2106a 2106b 2107b 2105	2106r 2106a 2106b 2107b 2105	2106r 2106a 2106b 2107b 2105

C= cypermethrin, A= Acetochlor

The study found that natural selection is responsible for diversity of xenobiotic degradation shown by a lot of diversity in Nkhozho and Mulanje which has long history of pesticide application compared to CHAMPHOYO farm in Karonga which had a year of application as shown in table 2. These results also show that aerial application has an impact to non-target sites shown by diversity of microbes responsible for degrading xenobiotics outside the farm (table 2).

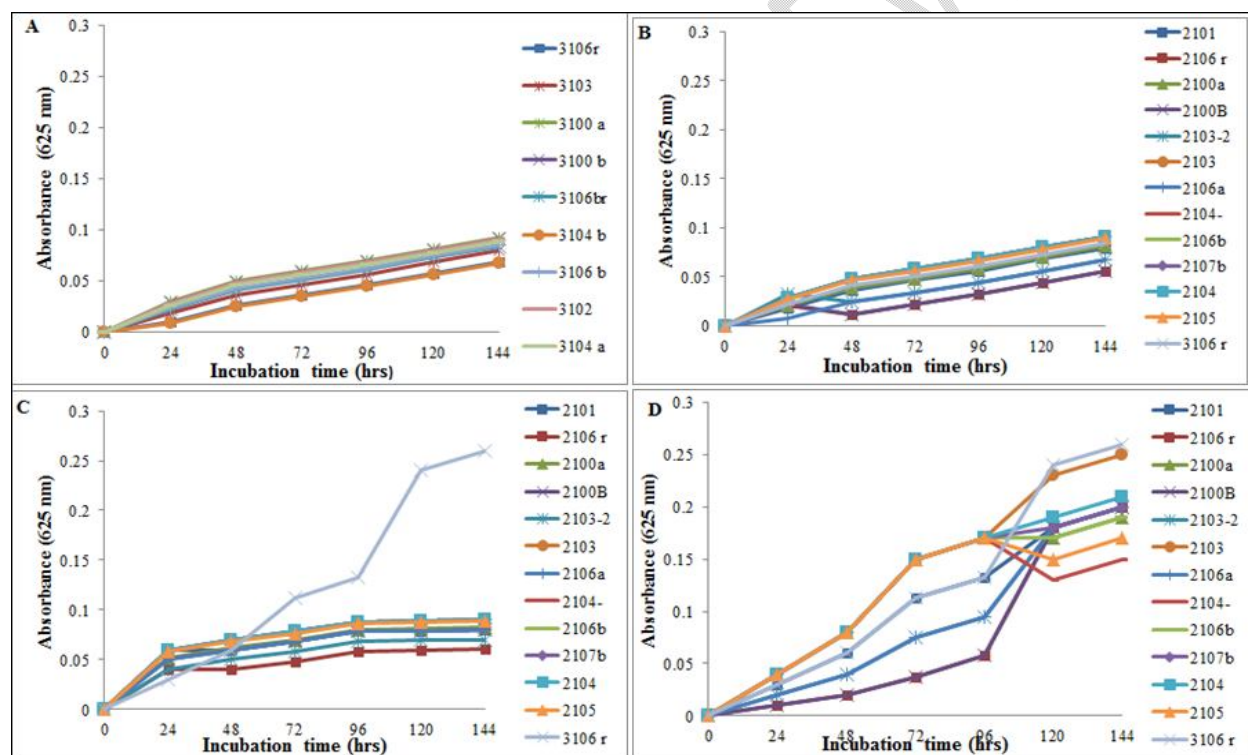


Figure 1: Growth kinetics of cypermethrin and acetochlor degrading bacteria through utilization of pesticides as sole carbon and phosphorous source for 144 hours. A: Growth kinetics of isolates using cypermethrin as sole carbon source, B: Growth kinetics of isolates using acetochlor as sole carbon and P source, C: Growth kinetics of isolates in acetochlor as sole phosphorus source, D: Growth kinetics of isolates using Acetochlor as sole carbon source.

Results on growth kinetics show that different isolated microorganism showed different growth rates under different conditions. Microbes show high growth rate by utilisation of acetochlor as carbon source than Phosphorous source as shown in figure 1 B and 2 D. isolates showed slow growth rate in utilisation of cypermethrin than in acetochlor as carbon sources as shown in figure 1 A and 2 D. By utilising carbon

from pesticide they are degrading the pesticide using laccase enzyme. All microbes that were able to utilize cypermethrin and acetochlor showed the presence of laccase enzyme as also indicated on table 1.

Table 3: Isolates and their blast related species and GenBank deposit accession numbers

LAB NO	RELATED SPECIES	NUCLEOTIDE IDENTITY %	ACCESSION NUMBER
3106b	<i>Enterobacter asburiae</i>	99	MF979777
3106 br	<i>Enterobacter asburiae</i>	99	MF979662
2106b	<i>Enterobacter cloacae</i>	99	MF979810
2106A	<i>Enterobacter cloacae</i>	97	MF979821
2105	<i>Enterobacter sp.</i>	91	MF979964
2104-2	<i>Enterobacter sp.</i>	99	MF979876
2104-1	<i>Enterobacter cloacae</i>	99	MF979885
2103	<i>Enterobacter sp.</i>	99	MF980152
2103-2	<i>Enterobacter sp.</i>	99	MF980711
2101	<i>Enterobacter sp.</i>	99	MF980718
3106B	<i>Enterobacter sp.</i>	98	MF980912
2107b	<i>Pantoea agglomerans,</i>	96	MF980788
2100a	<i>Enterobacter cloacae</i>	99	MF980882
2100B	<i>Enterobacter sp.</i>	99	MF980911
3103	<i>Enterobacter asburiae</i>	98	MF980919
3100 a	<i>Enterobacter cloacae</i>	99	MF980916
3100 b	<i>Klebsiella pneumoniae</i>	99	MF980917
3106 r	<i>Enterobacter asburiae</i>	99	MF980922
3104 b	<i>Klebsiella pneumoniae</i>	98	MF980921
3102	<i>Serratia marcescens</i>	98	MF980918
3104 a	<i>Pseudomonas aeruginosa</i>	93	MF980920

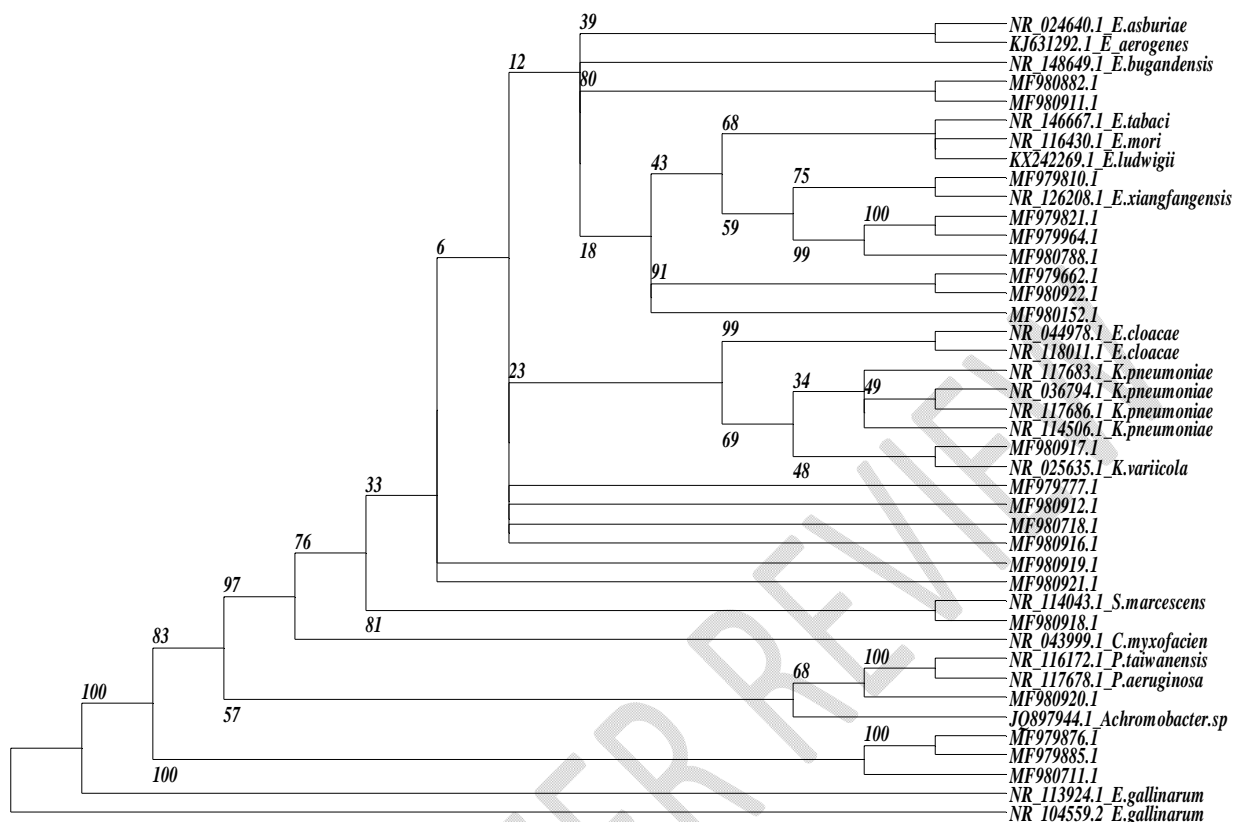


Figure 2: Phylogenetic tree based on 16S rDNA gene sequence derived from maximum parsimony analysis showing the position of cypermethrin and acetochlor degrading microbes isolated from selected agro ecological zones of Malawi and those of NCBI. Bootstrap values (%) are indicated at the nodes. Those with accession numbers with no names attached are from different agro ecological zones of Malawi as indicated on table 1, and 3 while the corresponding ones attached to the name are obtained from GenBank.

4. DISCUSSION

Potentiality of bioremediation require complex interaction knowledge of biochemical, physiological, ecological, microbiological, and molecular aspects involved in xenobiotics degradation¹³. The study found out that some microbes can utilize pesticides as sole carbon or P source or both as shown in table 1 which is in line with other reports^{22,32}. In this study only bacteria were found to degrade cypermethrin and acetochlor and some microbes were found to degrade both cypermethrin and acetochlor like *Enterobacter asburiae* table 1. Genus *Enterobacter* domination in bioremediation is in line with other studies^{7,33,34}.

The study also puts proposition that pest infestation in fields where pesticide application is a result of abundance of xenobiotic degrading microbes due to natural selection pressure not pesticide resistance by the pest table 2³⁵.

Higher hydrophobicity causes strong adsorption to soil of synthetic pyrethroid insecticides cypermethrin not leached³⁶. Half-life of this pesticide vary from 4 days to 8 weeks and is significantly affected by soil characteristics and microbial activity³⁷. The major degradation pathway of cypermethrin is 3-phenoxy benzyl alcohol and 3-phenoxy benzoic acid which acts hydrolytically by cleaving of an ester linkage. 3-phenoxy benzoic was observed by presence of layers on degrading tubes. Several microbes were isolated for degradation of cypermethrin by utilization of cypermethrin as sole carbon source which concur

with other studies^{22,38}; *Serratia marcescens* and *Pseudomonas*^{39,40}, *Enterobacter*^{7,41,42} but no study has found *Leclercia* sp and *Klebsiella pneumoniae* strains that can degrade cypermethrin. Thus, this finding adds strains of *Leclercia* sp, *Klebsiella pneumoniae* etc. to the list of cypermethrin degrading microbes.

However, there are reports that some of above isolated microbe can degrade other pesticides like glyphosate and others, but no study has documented that these strains degrade glyphosate in Malawi²¹. The study also found that all microbes were not able to utilize cypermethrin as sole P source. This could be because P is not active ingredient of cypermethrin and its adjuvants.

Strong mobility of Acetochlor poses an environmental risk to arable land, groundwater and surface water⁴³. Acetochlor is also suspected to be endocrine disruptor and regarded as a probable human carcinogen. Half-lives of the acetochlor are 3.4 and 2.8 days in the bulk soil and rhizosphere respectively but residue of 0.02–0.07 µg/g can still be detected 40 days after its application in the soil but do not confer a long term impairment on viable bacterial groups⁴⁴. All microbes were isolated for degradation of acetochlor by utilization of acetochlor as sole carbon source which is in support of other related studies of^{22,38}. One strain *Enterobacter asburiae* was found to degrade both acetochlor and cypermethrin which was found in 2 sites which is in support of studies that associate the microbe with degradation of acetochlor^{45,46}.

All isolates had shown laccase production ability using SDA amended with ABTS. Results concur with other studies that document potential applications of laccases are related to bioremediation and waste treatment like degradation and detoxification of pollutants^{47,48}. Laccase also plays important roles in, lignolytic degradation, detoxification studies, plant pathogenesis, odor control in decomposition of wastes and pigment production⁴⁸. Xenobiotics induce laccase gene transcription because of its interaction with receptors in the promoter regions of the genes encoding for laccase⁴⁹.

Beside xenobiotics degrading these isolates can be used to support growth and development of crop plants because of production of multiple PGP like Phosphate solubilization IAA, Siderophore, Catalase etc.as shown in table 1⁵⁰. Some of these phosphate solubilising microbes (PSM) like, *Klebsiella pneumoniae* have been documented antifungal activity towards *Fusarium oxysporum*, *Sclerotium rolfsii*, *Alternaria alternatae* and *Macrophomina phaseolina*⁵¹ while some might also enhance drought tolerance in plant.

Phylogenetic analysis based on the ML methods revealed that diversified divergent genera and species are involved in degradation of acetochlor and cypermethrin shown by bootstrap values as shown in figure 1. Genus *Enterobacter* is dominating in terms of diversity at species level and strain level in degradation of acetochlor and cypermethrin.

Phylogenetic tree shows diversity of isolates from Malawi forming unique clades separate from those of GenBank of NCBI. More than 95% of isolate unique clades were formed by those degrading Acetochlor herbicide based on agro ecological zone indicating that they have distant relationship. Many isolates formed single outgroup clades like isolate MF980152 or as group outgroup clade MF979876, MF979885 and MF980711.

5. Conclusion

The research met its objective in identifying indigenous strains involved in degradation of cypermethrin and Acetochlor. The study also revealed new strain of microbes involved in degradation of cypermethrin and Acetochlor and the study puts proposition that pest infestation in fields is a result of abundance of xenobiotic degrading microbes due to natural selection pressure not pesticide resistance of the pest.

REFERENCES

1. Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V. Microbial & Biochemical Technology Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. *J Microb Biochem Technol*. 2015;7(7):96-102. doi:10.4172/1948-5948.1000188

2. Jhala AJ, Malik MS, Willis JB. Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean. Published online 2015. doi:10.4141/CJPS-2014-422
3. Fisher PR, Appleton J, Pemberton JM. Isolation and Characterization of the Pesticide-Degrading Plasmid pJP1 from *Alcaligenes paradoxus*. 1978;135(3):798-804.
4. 1.ISCA-RJRS-2017-087(1).
5. Kearney PC (Philip C., Roberts TR (Terence R. *Pesticide Remediation in Soils and Water*. Wiley; 1998.
6. Kucharski M, Dziągwa M, Sadowski J. Monitoring of acetochlor residues in soil and maize grain supported by the laboratory study. 2014;60(11):496-500.
7. Thatheyus AJ, Selvam ADG. Synthetic Pyrethroids : Toxicity and Biodegradation. 2013;1(3):33-36. doi:10.12691/aees-1-3-2
8. Ahmad F, Ā IA, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. 2008;163. doi:10.1016/j.micres.2006.04.001
9. Johal GS, Huber DM. Glyphosate effects on diseases of plants. Published online 2009. doi:10.1016/j.eja.2009.04.004
10. Beres KK. The toxicity of glyphosate - based herbicides to soil bacteria and freshwater crustaceans. 2016;(September).
11. Eman A, Abdel-megeed A, Suliman A meshal A, Sadik MW. Original Research Article Biodegradation of Glyphosate by fungal strains isolated from herbicides polluted-soils in Riyadh area. 2013;2(8):359-381.
12. Carvalho FP. Pesticides , environment , and food safety. Published online 2017. doi:10.1002/fes3.108
13. Singh R, Singh P, Sharma R. Microorganism as a tool of bioremediation technology for cleaning environment : A review. 2014;4(1):1-6.
14. Annibale AD, Rosetto F, Leonardi V, Federici F, Petruccioli M. Role of Autochthonous Filamentous Fungi in Bioremediation of a Soil Historically Contaminated with Aromatic Hydrocarbons. 2006;72(1):28-36. doi:10.1128/AEM.72.1.28
15. Ahuja SK, Ferreira GM, Moreira AR, Ahuja SK, Ferreira GM, Utilization ARM. *Critical Reviews in Biotechnology Utilization of Enzymes for Environmental Applications Utilization of Enzymes for Environmental*. Vol 8551.; 2016. doi:10.1080/07388550490493726
16. Barot J, Chaudhari K. Analysis of Dimethoate Degradation By *Kocuria Turfanensis* Using Gc-Ms. *Asian J Microbiol Biotechnol Environ Sci*. 2020;22(July):107-110.
17. Ramganga UP, Sarah R, Tabassum B, et al. Saudi Journal of Biological Sciences Bioaccumulation of heavy metals in *Channa punctatus* (Bloch) in river. *Saudi J Biol Sci*. 2019;26(5):979-984. doi:10.1016/j.sjbs.2019.02.009
18. Benelli G. Saudi Journal of Biological Sciences Managing mosquitoes and ticks in a rapidly changing world – Facts and trends. *Saudi J Biol Sci*. 2019;26(5):921-929. doi:10.1016/j.sjbs.2018.06.007
19. Sundaram SK, Taj NA, Menaka R, Ajitha C. Biodegradation of Pesticides Through Metagenomic Approaches. *J Adv Sci Res*. 2022;13(02):29-37. doi:10.55218/jasr.202213205

20. Derbalah A, Massoud A, El-Mehasseb I, et al. Microbial detoxification of dimethoate and methomyl residues in aqueous media. *Water (Switzerland)*. 2021;13(8):1-14. doi:10.3390/w13081117
21. Mwafulirwa S, Obiero G, Mpeketula P, Aboge G. Isolation of indigenous glyphosate degrading microbes from selected agro ecological zones of Malawi. 2017;6(12):1-9.
22. Shamsuddeen U, Inuwa AB. Utilization Of Cypermethrin By Bacteria Isolated From Irrigated Soils. 2013;6(2):19-22.
23. Akbar S, Sultan S, Kertesz M. Determination of Cypermethrin Degradation Potential of Soil Bacteria Along with Plant Growth-Promoting Characteristics. Published online 2015:75-84. doi:10.1007/s00284-014-0684-7
24. Singh N, Abraham J. Isolation of laccase producing fungus from compost soil and partial characterization of laccase. 2013;4(5):91-98.
25. Ambreen S, Yasmin A. Isolation, characterization and identification of organophosphate pesticide degrading bacterial isolates and optimization of their potential to degrade chlorpyrifos. *Int J Agric Biol*. 2020;24(4):699-706. doi:10.17957/IJAB/15.1489
26. Singh S, Kumar V, Singla S, et al. Kinetic study of the biodegradation of acephate by indigenous soil bacterial isolates in the presence of humic acid and metal ions. *Biomolecules*. 2020;10(3):1-11. doi:10.3390/biom10030433
27. Aguila-Torres P, Maldonado J, Gaete A, et al. Biochemical and Genomic Characterization of the Cypermethrin-Degrading and Biosurfactant-Producing Bacterial Strains Isolated from Marine Sediments of the Chilean Northern Patagonia. *Mar Drugs*. 2020;18(5). doi:10.3390/md18050252
28. Saied E, Fouda A, Alemam AM, et al. Evaluate the toxicity of pyrethroid insecticide cypermethrin before and after biodegradation by *lysiniabacillus cresolivuorans* strain his7. *Plants*. 2021;10(9). doi:10.3390/plants10091903
29. Karafakiog YS. Effects of a lipoic acid on noise induced oxidative stress in rats. 2019;26:989-994. doi:10.1016/j.sjbs.2018.08.008
30. S SN, Gomathy M, Manikandan K, Suresh S. Research Article Isolation And Characterization Of Zinc Solubilizing Bacteria From Soils Of Thoothukudi District. 2019;11(6):1620-1623.
31. Liu M, Liu X, Cheng B sen, et al. Selection and evaluation of phosphate-solubilizing bacteria from grapevine rhizospheres for use as biofertilizers. 2016;14(4).
32. Lim CJ. Glyphosate Utilization as the Source of Carbon: Isolation and Identification of new Bacteria. 2011;8(4):1582-1587.
33. Ogot HA, Boga HI, Budambula N, et al. Isolation , characterization and identification of roundup degrading bacteria from the soil and gut of *Macrotermes michaelseni*. 2013;1(1):31-38.
34. Kryuchkova Y V, Burygin GL, Gogoleva NE, et al. Isolation and characterization of a glyphosate-degrading rhizosphere strain , *Enterobacter cloacae* K7 & . *Microbiol Res*. 2014;169(1):99-105. doi:10.1016/j.micres.2013.03.002
35. Neumann D, Heuer A, Hemkemeyer M, Martens R, Tebbe CC. Importance of soil organic matter for the diversity of microorganisms involved in the degradation of organic pollutants. *ISME J*. 2014;8(6):1289-1300. doi:10.1038/ismej.2013.233
36. Kumari K, Fulekar DMH. Investigation of potential rhizospheric isolate for cypermethrin degradation. Published online 2013:33-43. doi:10.1007/s13205-012-0067-3

37. Bhosle NP, Khan ZS, Nasreen S, Road C. Manuscript Info. 2013;1(2320):229-238.
38. Bhosle NP, Nasreen S. Remediation of Cypermethrin-25 EC by Microorganisms Siddharth Art , Commerce and Science College Jafrabad , Dist Jalna. 2013;3(1):144-152.
39. Rani MS, Lakshmi KV, Devi PS, et al. Isolation and characterization of a chlorpyrifos- degrading bacterium from agricultural soil and its growth response. 2008;(2):26-31.
40. Malatova K, Morrill T. Isolation and characterization of hydrocarbon degrading bacteria from environmental habitats in Western New York State. Published online 2005.
41. Massiha A, Issazadeh K. Biodegradation of Cypermethrin by using Indigenous Bacteria Isolated from Surface Soil. 2012;35:71-76.
42. Roy O, Subbaiah UM. Optimization of Cypermethrin Degradation By Bacterial Cultures Isolated From Soil. 2017;02(01):299-305.
43. Borowik A, Kucharski J. Microbial and enzymatic activity of soil contaminated with a mixture of diflufenican + mesosulfuron-methyl +. Published online 2015:643-656. doi:10.1007/s11356-014-3395-5
44. Bai Z, Xu H juan, He H bo, Zheng L chen. Alterations of microbial populations and composition in the rhizosphere and bulk soil as affected by residual acetochlor. Published online 2013:369-379. doi:10.1007/s11356-012-1061-3
45. El-nahhal Y, Awad Y, Safi J. Bioremediation of Acetochlor in Soil and Water Systems by Cyanobacterial Mat. 2013;2013(July):880-890.
46. Martins PF, Carvalho G, Gratão PL, et al. Effects of the herbicides acetochlor and metolachlor on antioxidant enzymes in soil bacteria. *Process Biochem.* 2011;46(5):1186-1195. doi:10.1016/j.procbio.2011.02.014
47. Ck H, Gayathri V. Effect of Pesticide (Chlorpyrifos) on Soil Microbial Flora and Pesticide Degradation by Strains Isolated from Contaminated Soil. 2013;4(2):2-7. doi:10.4172/2155-6199.1000178
48. Viswanath B, Rajesh B, Janardhan A. Fungal Laccases and Their Applications in Bioremediation Fungal Laccases and Their Applications in Bioremediation. 2016;(October). doi:10.1155/2014/163242
49. Castilho FJD, Torres RA, Barbosa AM, Dekker RFH, Garcia JE. On the diversity of the laccase gene: A phylogenetic perspective from botryosphaeria rhodina (Ascomycota: Fungi) and other related taxa. *Biochem Genet.* 2009;47(1-2):80-91. doi:10.1007/s10528-008-9208-0
50. Asnawati F, Citra H, Handayani NI, et al. Ability Of Ammonium Excretion , Indol Acetic Acid Production , And Phosphate Solubilization Of Nitrogen-Fixing Bacteria Isolated From Crop Rhizosphere And Their Effect On Plant Growth. 2016;11(19):11735-11741.
51. Jahangir GZ, Sadiq M, Hassan N, Nasir IA, Saleem MZ, Iqbal M. The Effectiveness Of Phosphate Solubilizing Bacteria As Biocontrol Agents. 2016;26(5):1313-1319.