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DEGRADATION OF CYPERMETHRIN BY *PSYCHROBACTER* SP. AB2 ISOLATED FROM CYPERMETHRIN-TREATED AGRICULTURAL SOILS IN LAGOS, NIGERIA

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ABSTRACT
The ecological safety and health risk of cypermethrin is an emerging concern due to its carcinogenic, neurotoxic, and endocrine-disrupting effects on non-target organisms. The specific objective of this study was to isolate and characterize bacterial strains from cypermethrin-treated agricultural soils that are able to utilize cypermethrin as the sole source of carbon and energy. *Psychrobacter faecalis* strain AB2 was isolated using the aerobic enrichment culture technique. Degradation of cypermethrin and release of 3-Phenoxybenzoic acid (3-PBA) were detected using gas chromatography (GC) and reversed-phase high-performance liquid chromatography (HPLC). HPLC measurements of the culture supernatants showed that hydrolytic cleavage of the ester bond in the cypermethrin molecule resulted in the release of 3-PBA up to the level of 15.4 % by strain AB2 during growth on MSM supplemented with cypermethrin. Based on the result of 16S rRNA gene sequencing, the isolated bacterial strain was identified as *Psychrobacter faecalis* strain AB2 (KF688134). Strain AB2 exhibited a growth rate of 0.025 h^{-1} and a doubling time of 27.79 h^{-1} during growth in MSM supplemented with cypermethrin. Microcosm experiments showed the percentage of cypermethrin degradation was 60.19 % by strain AB2 after 20 days of incubation. Strain AB2 utilized a range of aromatic compounds as carbon sources, indicating its potential for bioremediation of a polluted environment.

Keywords: Cypermethrin, Ecological safety, Health risk, Carcinogenic effects, Neurotoxic effects, Endocrine disruption

INTRODUCTION
Pesticides are one of the most potentially harmful chemicals deliberately introduced into the environment through agricultural practices. An ideal pesticide should be toxic only to target organisms, biodegradable, and undesirable residues should not affect non-target organisms. Synthetic pyrethroids have been widely used as home and garden insecticides since 1978 (Kumar et al., 2010). With such extensive use, the accumulation of synthetic pyrethroids in the environment is bound to be substantial. Pesticide residues in soil can diffuse, evaporate, or leach, thus having the potential to cause water pollution and other ecological problems (Anozie et al., 2017). Although most synthetic pyrethroids have lower mammalian toxicity compared to other classes of insecticides (e.g., organochlorines or organophosphates), they can still be very harmful to certain vertebrate and mammal species, including chicks and fish or shellfish (Agwu et al., 2016; Alejandra, 2023; Kumar et al., 2023). Previous studies have been carried out to assess the effects of insecticides on microbial communities in different environments. Over 90 % of the cypermethrin manufactured worldwide is used to combat pests feeding on cotton crops.

Cypermethrin [RS-a-cyano-3-phenoxybenzyl (1RS)-cis-, trans-3-(2,2,- dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] is a synthetic pyrethroid which embodies three chiral centres, two in the cyclopropane ring and one on the α-cyano carbon, resulting in several stereoisomers. Cypermethrin is the racemic mixture (ratio 50:50) of all eight isomers [7, 9]. The α-cyano derivatives of pyrethroids (cypermethrin and decamethrin) are extremely active insecticides (Kosimov et al., 2025). Cypermethrin is an active ingredient in many formula-grade insecticides named as Colt, Ammo, Avicide, Barricade, CCN 52, Cymbush, Folcord, Imperator, Kafil Super, Polytrin, Ripcord, and Stockade. It is available as an emulsifiable concentrate or wettable powder. It has a very low vapour pressure and water solubility, and its moderately high partition coefficients (log Kow and log Koc values range from 4.47-6.3 and 3.3-5.0, respectively) indicate that it is strongly adsorbed from aqueous solutions by sediments and suspended particulate matter.

In Nigeria, pesticides constitute an important component of agricultural development and protection of public health since the tropical climate is very conducive to pest breeding. The main use of cypermethrin in Nigeria is for the protection of crops such as cucumber, pumpkin, maize, and watermelon against insect pests. The use of pyrethroid insecticides has increased over the years in Nigeria without adequate regulatory measures on the usage due to restrictions on organochlorinated insecticides such as DDT. It has been estimated that about 125,000-130,000 tons of pesticides are applied every year in Nigeria for the protection of insect pests from crops (Thatheyus et al., 2013). These pesticides, when discharged by agricultural run-off or spray drift and in aquaculture operations, are washed into nearby water bodies and can thereby affect susceptible non-target organisms such as fish, prawn (Agwu et al., 2016; Alejandra, 2023), and honey bees (Mazi et al., 2020).

Microbes play significant roles in degrading and detoxifying pyrethroid residues in the environment. The primary mechanism for the dissipation of cypermethrin from the environment is through biological processes involving cleavage of the ester linkages, to give the two main degradation products, 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane-carboxylic acid (CPA) and 3-phenoxybenzoic acid (PBA) (Thatheyus et al., 2013). Under

aerobic conditions, these metabolites may undergo further breakdown to CO_2 at a much slower rate.

Pyrethroid-degrading bacteria such as *Achromobacter* sp., *Bacillus cereus*, *Pseudomonas fluorescens, Serratia plymuthica, and Pseudomonas* sp. YFO5, *Micrococcus* sp., *Ochrobactrum anthropi* JCm1, *Bacillus megaterium* JCm2, *Rhodococcus* sp. JCm5, *Acinetobacter calcoacetius* MCm5, *Brevibacillus parabrevis* FCm9, and *Sphingomonas* sp. RCm6 have been isolated and characterized as cypermethrin degraders (Cycoń & Piotrowska-Seget, 2016).

The fate of pesticides under normal environmental conditions is controlled mainly by temperature, humidity, organic matter content, and light intensity (Kosimov et al., 2025). It is not possible to predict their fate under the environmental conditions of Nigeria, which is known for high and low temperatures during dry and rainy seasons, respectively. This is particularly true for cypermethrin, which is widely used in Nigeria, and a literature search indicates that the effects of this pesticide on tropical soil microorganisms and their activities are very scarce.

Thus, this study aimed to isolate and characterize competent cypermethrin-degrading microorganisms from tropical agricultural soil and evaluate their potential in bioremediation of cypermethrin and other aromatic pollutants in the environment.

MATERIALS AND METHODS

Soil Sample Collection

Soil samples used in this experiment were collected from three different agricultural fields of Gbodo in Ikorodu Local Government Area in Lagos State, Nigeria, which had a history of CPM application for more than 10 years. Samples were collected from 5 to 20 cm depth, pooled together, homogenized, and immediately placed in sterile plastic bags and transported to the laboratory. Samples for chemical analysis were stored at -2 ^oC while soil samples for microbiological analysis were stored at 4 ^oC for future use. All microbiological analysis was performed within two days of sampling.

Chemicals and Media

Technical grade cypermethrin (CYP, 96%) and 3-Phenoxybenzoic acid (3-PBA) ($C_{13}H_{10}O_3$, CAS NO: 3739-38-6, EC NO: 223-121-2) were obtained from Sigma-Aldrich Chemical Co., South Africa. Stock solution of CYP was prepared by dissolving 100 mg of technical grade CYP in 1 ml of acetone, and this solution was kept at 4 ^oC before use. Methanol and acetonitrile were of HPLC (high-performance liquid chromatography) purity grade. Orthophosphoric acid (85%, purity) was obtained from Fluka Chemical Company. All other chemicals were analytical grade, ≥ 98 % purity.

Cypermethrin-degrading bacterial strain was isolated in MSM (pH 6.80-7.00) containing (g L^{-1}) $Na_2HPO_4.2H_2O$, 3.5; KH_2PO_4, 1; $(NH_4)2SO_4$, 0.5; $MgCl_2.6H_2O$, 0.1; $Ca(NO_3)_2.4H_2O$, 0.05, and 0.5 ml of trace element solution. The trace element was composed of (mg L^{-1}) EDTA, 5000; $Fe(II)SO_4.7H_2O$, 3000; $MnCl_2.4H_2O$,30; $Co(II)Cl_2.6H_2O$, 50; $CuCl_2.2H_2O$, 10; $NiCl_2.6H_20$, 20; $Na_2MoO_2.2H_2O$, 30; $ZnSO_4.7H_2O$, 50 and H_3BO_4, 20. For medium solidification, 1.5% of agar was used.

Enrichment, isolation, and screening of cypermethrin-degrading microorganisms

Cypermethrin-utilizing indigenous to the soils were isolated by enrichment culture technique as described by Gottsching and Schmidt (2007). Soil sample (1g) was added to 99 ml of mineral salts medium (MSM), which contained 10 mg of analytical grade cypermethrin in a 250 ml Erlenmeyer flask. The flask was incubated at 28 ^oC on a rotary shaker at 180 rpm for the first four weeks. Thereafter, enrichment cultures were transferred to a fresh MSM using 1% inocula and 10 mg analytical grade cypermethrin. Cultivation of the enrichment cultures continued under the same conditions. This procedure was repeated seven consecutive times, and transfers were made to a fresh MSM every two weeks. Utilization of cypermethrin in the enrichment medium resulted in a visual increase in the turbidity of the medium and a gradual disappearance of the compound over time. Pure cultures were subsequently isolated from the enrichment medium by the spread-plate technique. Aliquots (0.1 ml) of enrichment medium were plated on cypermethrin MSM agar. Plates were incubated at 28 ^oC for 2 to 5 days. Predominant colonies appearing on the dilution plates after five days of incubation were isolated and purified on cypermethrin MSM agar.

Identification and Characterization of the Organism

Identification of bacterial isolate was carried out using standard cultural, morphological, and biochemical characteristics according to the method of Cowan and Steel (Barrow and Feltham, 1995). *Pseudomonas* sp. The American Type Culture Collection was used as a control for the biochemical test. The identification of the isolate was complemented using molecular biology techniques. Molecular identification of the bacterial isolate was done by the sequencing of the PCR amplified 16S rRNA gene. Briefly, the genomic DNA of the isolate was extracted by a simple freeze-thaw method. Isolates were grown on nutrient agar for 18 to 24 h. Thereafter, a colony of the isolate was suspended in 100 µl of sterile distilled water or buffer (1 x Tris-acetate or Tris-borate) in a 1.5 ml of micro centrifuge tube. The suspension was boiled for 10 min at 85 ^oC in a heating block, followed by 3 min of freezing in liquid nitrogen. Five freeze-thaw cycles were employed to enhance DNA extraction. The samples were then centrifuged at 12,000 rpm for 3 min, and the supernatant, which contained the DNA, was transferred to a clean microcentrifuge tube. Then, 1 µl of the supernatant (DNA template) was used for PCR analysis.

The 16S rRNA gene sequence was amplified using the forward primer 27F (5'- AGAGTTTGATCCTGGCTCAG -3') and reverse primer 1492R (5'- GGTTACCTTGTTACGACTT -3'). The PCR reaction was carried out by subjecting 1 µl of the genomic DNA to PCR in a final reaction volume of 25 µl. The PCR mixtures contained 0.6 µl each of reverse and forward primer; 2.5 µl of $MgCl_2$; 0.5 µl of dNTP (deoxynucleoside triphosphate); 5 µl of buffer (KapaTaq Hotstart Buffer); 0.2 µl of Taq polymerase; 14.6 µl of MilliQ water, and 1 µl of template DNA. All the PCR reagents were purchased from Kapa Biosystems, Massachusetts, United States. Amplification was performed in a thermocycler (Eppendorf Mastercycler) programmed as follows: initial denaturing temperature cycle at 94 ^oC for 2 minutes, 35 cycles of denaturation at 95 ^oC for 30 sec, annealing at 55 ^oC for 30 sec and extension at 72 ^oC for 1 min. A final extension cycle was carried out at 72 ^oC for 7 min (Khaled et al., 2012). The temperature within the thermocycler was held at 4 ^oC until the tubes were stored in a refrigerator at -20 ^oC until future use. The size of the amplification product was verified by gel electrophoresis (1% agarose, Tris-Borate buffer pH 8.0). The purified DNA was sequenced at Inqaba Biotechnologies, Pretoria, South Africa. The sequence obtained

was aligned using the Basic Local Alignment Search Tool (BLAST, www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) and compared with small-subunit (SSU) sequences deposited with NCBI GenBank (www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (www.rdp.cme.msu.edu). MEGA (v. 6.0) was employed to construct a phylogenetic tree based on the neighbour joining method using type strain sequences obtained from the RDP (Tamura et al., 2011) with re-sampling for 1000 replicates.

Biodegradation Studies

Inoculum Development

Inocula used for cypermethrin utilization studies were pre-grown on 50 ml of mineral salts medium (MSM) supplemented with cypermethrin (final concentration of 100 mg L^{-1}) in 250 mL Erlenmeyer flasks and incubated for 24 h on bench top orbital shaker (180 rev min^{-1}) at 28 ^{o}C. After incubation, cultures were harvested by centrifugation at 12,000 rev min^{-1} for 5 min and washed twice with sterile MSM. Bacterial cells were re-suspended in sterile MS medium, the optical density was adjusted to 0.5, and used for biodegradation studies (Hu et al., 2019).

Cypermethrin Utilization Studies

Prior to inoculation, a 5 mg stock solution of active cypermethrin (at a final concentration of 100 mg L^{-1}) was aseptically transferred into a sterile 250 mL Erlenmeyer flask. The flasks were placed on an orbital shaker (180 rev min^{-1}) at 28 ^{o}C for 24 h in a laminar flow to achieve complete evaporation of the acetone. After evaporation of acetone, 50 ml of MSM was added, and the flask was placed back on an orbital shaker for 30 minutes to achieve complete homogenization of the medium. Pre-grown washed cells (1 %, v/v) were aseptically inoculated into the medium and incubated under the same conditions. Starting from time zero (immediately after inoculation) and every 24 h interval, 1 ml aliquot of the utilizing culture samples was aseptically withdrawn and centrifuged at 12,000 rpm for 10 min for HPLC analysis. The supernatant was carefully transferred into 1.5 ml Eppendorf tubes and stored at -20 ^{o}C until used for HPLC analysis of metabolites.

For growth studies, 1 ml of the culture medium was withdrawn, and optical density at 600 nm was measured spectrophotometrically in a UV-VIS spectrophotometer. Total cell counts were obtained by using a bacterial counting chamber (Hawksley Helber, dimensions: 0.00025 x 0.02 mm). Residual cypermethrin concentration was assayed using GC-FID. Duplicate control flasks were also set up alongside experimental flasks. The control flasks contained 100 mg L^{-1} of cypermethrin and heat-inactivated cells (80 ^{o}C, 30 minutes) to monitor abiotic factors in the degradation of cypermethrin (Hu et al., 2019).

Detection of Cypermethrin Degradation Metabolites

Detection of cypermethrin degradation metabolites from cell-free culture of strain AB2 grown in liquid MSM supplemented with cypermethrin (at a final concentration of 100 mg L^{-1}) was screened for the presence of cypermethrin metabolites by reversed phase HPLC (Varian Prostar, USA) with diode-array detection (DAD) at 210 and 270 nm. Authentic 3-PBA was used as a calibration standard. An Inertsil OD-3 C18 column (5 µm, 250 x 4 mm) was used (Schambeck, Germany) with isocratic elution using a mobile phase consisting of 70:30 methanol: water acidified with 0.3 g L^{-1} o-phosphoric acid. Samples (1 ml) of cell-free culture supernatant (obtained by centrifugation at 12,000 rpm for 10 min) taken out periodically were analyzed directly. The metabolites were identified based on retention time and peak area of the pure standard (Anozie et al., 2017).

Degradation of cypermethrin in soil microcosm experiments

Microcosms were set up according to the method described by Sundaram et al. (2013) with some modifications. Soil samples were obtained from an uncontaminated site in the botanical garden at the University of Lagos. Soil was dried at 50 ^{o}C for 3 days, sieved through a 2-mm sieve, and sterilized in small foil packets by autoclaving at 121 ^{o}C for 30 min on three consecutive days. Triplicate soil samples (10 g) were placed in 150 mL beakers, and their water contents were adjusted to 60% of water holding capacity for each of the setups. Degradation analysis of cypermethrin was carried out in sterilized soil (SS) and non-sterilized soil (NSS) inoculated with strain AB2. Each soil sample was amended with cypermethrin dissolved in acetone to obtain a final concentration of 100 mg kg^{-1} of dry soil. To homogenize the cypermethrin with the soil, the soil samples were stirred using a sterilized spatula. After mixing and solvent evaporation, bacterial strain AB2 was inoculated into each of the soil samples. Non-inoculated sterilized soil (Ni-SS) samples served as the control. All the beakers were covered with perforated aluminium foil and then incubated at room temperature (26 ^{o}C ± 2 ^{o}C) for 20 days. Soil moisture content was maintained throughout the experimental period by the addition of sterile distilled water. After 0, 10, and 20 days, the entire content of soil samples from each beaker was extracted with a hexane/acetone mixture, and residual cypermethrin concentration was determined chromatographically using GC-FID analysis.

Extraction of residual cypermethrin from culture medium and soil microcosm for Gas Chromatography-Flame Ionization Detector (GC-FID)

Extraction of cypermethrin from culture medium and soil samples was carried out according to the method described by Sundaram et al. (2013) with some modifications. Soil samples (10 g) and culture medium (40 mL) were extracted three times with 50 mL of hexane/acetone mixture (1:1, v/v). After vigorous shaking for 20-30 min, the solvent layer was filtered, dried with dehydrated sodium sulfate, and allowed to evaporate at room temperature (26 ^{o}C ± 2 ^{o}C). The extract was dissolved with 1 ml of acetone, and an aliquot of 5 µL was used for GC-FID analysis.

An HP6890N (Agilent Technologies) powered gas chromatograph equipped with a flame ionization detector (GC-FID) was used for residual cypermethrin detection. Chromatographic separation was achieved by using a fused-silica capillary column (30 m x 0.23 mm id) coated with 5% phenylmethylpolysiloxane (0.25 µm). The injector was in the split mode (10:1), and its temperature was maintained at 270 ^{o}C throughout. The column temperature was raised from 180 ^{o}C (hold 1 min) to 220 ^{o}C (hold 2.00 min) at 5 ^{o}C for 5 min and then to 280 ^{o}C (hold 30 min) at 30 ^{o}C min^{-1}. Nitrogen was used as carrier gas at a flow-rate of 1.5 mL min^{-1}. The detection was carried out by a flame ionization detector with the temperature of 300 ^{o}C and the ratio of hydrogen/air is 40/400.

Cross-Feeding Studies

The ability of *Psychrobacter faecalis* strain AB2 to utilize other aromatic and non-aromatic compounds structurally related to cypermethrin was tested in MSM supplemented with selected

compounds as sole carbon and energy source at 2 mM L^{-1}. Incubation was carried out at room temperature in the dark for 10 days. Growth was verified by $OD_{600\ nm}$ measurements and visual observation for turbidity, which was not observed in control flasks. Cultures incubated in the absence of aromatic and non-aromatic compounds as the utilizable carbon source served to demonstrate that biomass formation depended on the utilization of these compounds, while incubations without active cells present served as abiotic controls (Moxley & Schmidt, 2010).

Transmission electron microscopy (TEM)

Cell morphology and flagellation were verified by transmission electron microscopy (TEM) (Philips, CM 120 Biotwin) using formvar-coated copper grids (Moxley & Schmidt 2010). A droplet of bacterial cells grown for 24 h was placed on parafilm, and a formvar-coated copper grid was inverted onto the droplet. After about 4-5 min (the particulates attached to the formvar primarily by electrostatic forces), the grid was wiped to almost dryness with Whatman's filter paper. The culture was stained with 2 % phosphotungstic acid for 30 sec and wick the grid to dryness with filter paper and examined under the TEM.

Scanning electron microscopy (SEM)

Scanning electron microscopy was carried out according to the method described by Hirsch & Christensen (1983). The bacterial colony was inoculated on Luria Bertani broth and incubated for 24 h. A drop of the culture broth was placed on a filter membrane (0.4-0.2 µm) and allowed to air dry. Thereafter, samples were fixed in 3 % glutaraldehyde for 45 min, rinsed 3 times in sodium cacodylate buffer (0.05 M, pH 7.2), and dehydrated with increasing concentrations of ethanol (from 10, 30, 50, 70, 80, 90, and 100 %) for 10 min at each rinse. After dehydration, the cells were dried in a critical point drier (Hitachi HCP-2). Specimens were affixed to aluminum stubs and sputter-coated with gold and palladium for 3 min. Samples were viewed by a Variable vacuum Environmental Scanning Microscope [ESEM] (Zeiss EVOLS15, USA).

Statistical Analysis

All statistical analysis was performed using SAS statistical software version 9.2 (Statistical Analysis Software Inc. 1990) and Prism computer software programmes (Graphpad Software, San Diego, CA, USA) version 5.03. Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) were used to test the significant difference at 5 % ($P < 0.05$) significance level.

RESULTS AND DISCUSSION

Cypermethrin-degrading bacterial strain AB2 was isolated from enrichment cultures inoculated with soil samples collected from agricultural farmland by using cypermethrin (at a final concentration of 100 mg L^{-1}) as the sole source of carbon and energy. The pH of the soil samples is 7.00. The soil samples were sandy loam in texture (sand 75.90 %, silt 21.08 %, and clay 1.59 %) with 11.92% moisture content, 1.09% total organic matter content, and 80 % water holding capacity.

Cypermethrin is an insecticide widely used to control pests in cotton and vegetable crops. Its use will continue to increase due to restrictions on the use of organophosphate pesticides (Kumar et al., 2023). Although these pyrethroid insecticides are considered safe for humans, studies abound that have shown that very high exposure to pyrethroids might cause potential problems to man such as a suppressive effect on the immune system (Deng et al., 2019; Alejandra, 2023). Therefore, biodegradation is a reliable, cost-effective technique for pesticide abatement and a major factor determining the fate of pyrethroid pesticides in the environment. Previous studies have shown that cypermethrin-contaminated sites are reservoirs of cypermethrin degraders (Hu et al., 2019; Alejandra, 2023). For instance, Akbar et al. (2015) and Zhang et al. (2010) isolated cypermethrin-degrading bacterial and fungal species from cypermethrin-contaminated agricultural farmlands.

Identification of cypermethrin degrading bacterial strain AB2

The physiological and biochemical characteristics of strain AB2 revealed that the organism was smooth, creamy, halophilic (tolerates 18 % Nacl concentration, data not shown), and gram-negative cells. *Psychrobacter faecalis* strain AB2 is catalase and oxidase positive, a characteristic observed in other *Psychrobacter* species such as *Psychrobacter sanguinis* sp. nov. (Wirth et al., 2012) and *Psychrobacter* spp. ORHg1, ORHg3 and ORHg8 (Pepi et al., 2011). It displays the ability to hydrolyse Tween 80 but does not hydrolyse urea, starch, and gelatin – a profile similar to that of *Psychrobacter aestuarii* sp. nov. (Baik et al., 2010). Scanning electron micrograph observations showed that the cells have cocci shape measuring about 1.2 x 0.7 µm in diameter (Fig. 1). Transmission electron micrograph revealed the absence of a flagellum by strain AB2, which conforms with the conventional features of *Psychrobacter* species (Fig. 2). Taxonomic assignment based on 16S rRNA gene sequence revealed that strain AB2 exhibited the highest sequence similarity (100 %) with an isolate of *Psychrobacter faecalis* (GenBank accession number AJ421528). The sequence obtained from strain AB2 was deposited in GenBank under accession number KF688134. *Psychrobacter* sp. 16S rRNA gene sequences for *Psychrobacter* sp. type strains were obtained from the Ribosomal Database Project, and a phylogenetic tree was constructed (Fig. 3). Based on morphological, physiological, and 16S rRNA gene sequence properties of strain AB2, the organism was assigned to the gammaproteobacteria genus *Psychrobacter faecalis* strain AB2. Members of the genus *Psychrobacter* have been isolated from a wide range of low temperature environments which include Antarctic sea ice, ornithogenic soil and sediments, the stomach contents of the Antarctic krill *Euphausia,* seawater (north-western Pacific Ocean, 300 m depth), the deep sea and the internal tissues of marine ascidian and crustacean species, low temperature Arctic permafrost, moderate-temperature marine environments, H_2O_2-containing wastewater (Baik et al., 2010) and polluted sediment of lagoon (Wirth et al., 2012). *Psychrobacter* sp. has also been isolated from pigeon faeces, fish, processed meat and poultry products, fermented seafood, and infected lamb (Baik et al., 2010). In this study, the isolation of *Psychrobacter faecalis* strain AB2 from tropical agricultural soil indicates that the ecophysiological versatility and biodiversity of these aerobic bacteria have not been fully elucidated yet, as new species were isolated from diverse environments.

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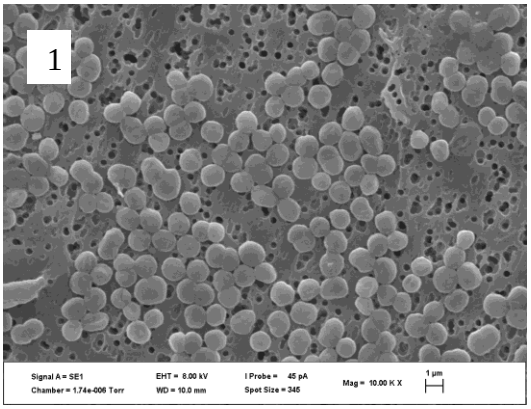

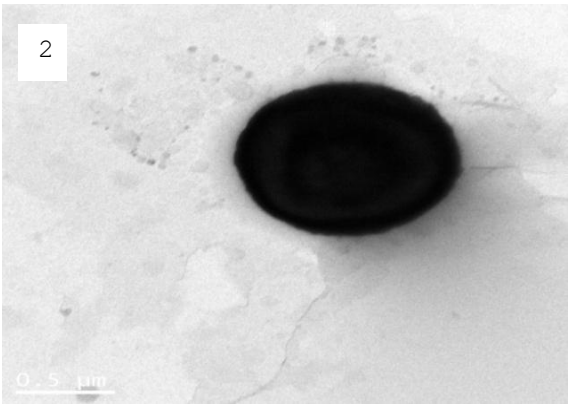

Fig. 1: Scanning electron micrograph of *Psychrobacter faecalis* strain AB2; Fig. 2: Transmission electron micrographs of a typical single cell of *Psychrobacter faecalis* strain AB2 showing a cocci-shaped cell. The inserted scale bar corresponds to 0.5 µm. Samples were viewed under high vacuum at a magnification of 18,000x.

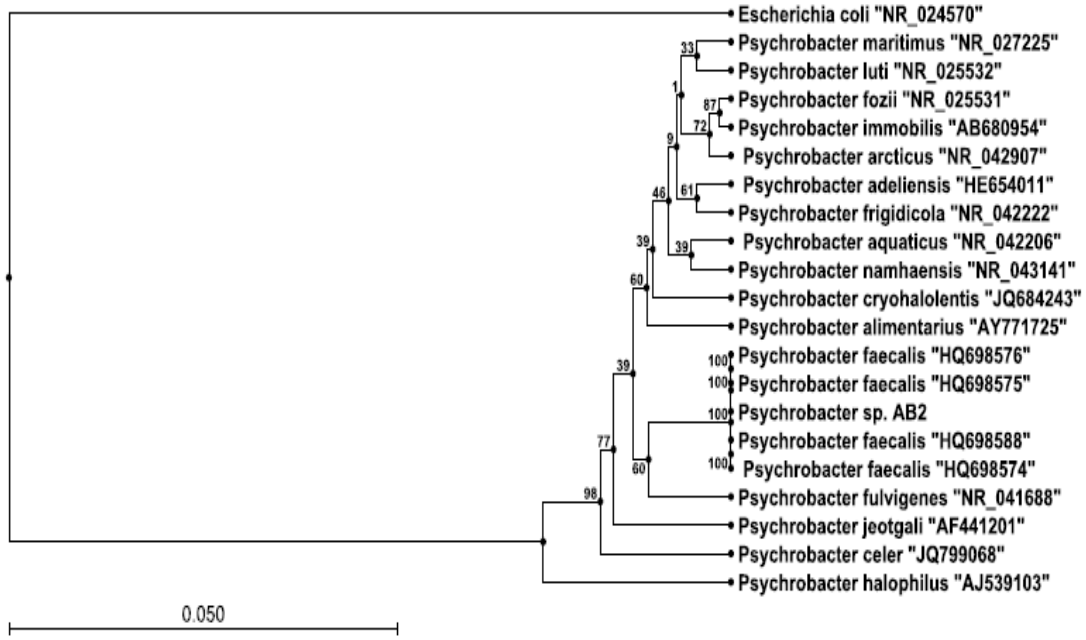

Fig. 3: Neighbour-joining tree and phylogenetic affiliation of *Psychrobacter faecalis* strain AB2 based on selected 16S rRNA gene sequences for type strains obtained from the Ribosomal Database Project. The 16S rRNA gene sequence of *E. coli* was used as an out-group.

Detection of metabolites in cell-free supernatant

HPLC measurements of the culture supernatants showed that hydrolytic cleavage of the ester bond in cypermethrin molecule resulted in non-stoichiometric release of 3-PBA (15.4 %) in the culture medium after 10 days growth of *Psychrobacter faecalis* AB2 on cypermethrin as only carbon and energy source (Fig. 4). The peak occurring at a retention time of 6 min corresponded with that of authentic standard under identical conditions. The insert shows heat-inactivated cells (incubated for 30 min at 80 ^{o}C) without detectable cleavage of cypermethrin. It is evident from HPLC results that strain AB2 degraded cypermethrin by hydrolysis of the carboxylester linkage to yield 3-phenoxybenzoic acid (Fig. 4). Similarly, microorganisms have been isolated to degrade pyrethroids through hydrolysis of the carboxylester bond in the compound. For example, Gottsching & Schmidt (2007), Guo et al. (2009), and Hu et al. (2019) reported degradation of cypermethrin by bacterial strains through hydrolysis of its ester linkage.

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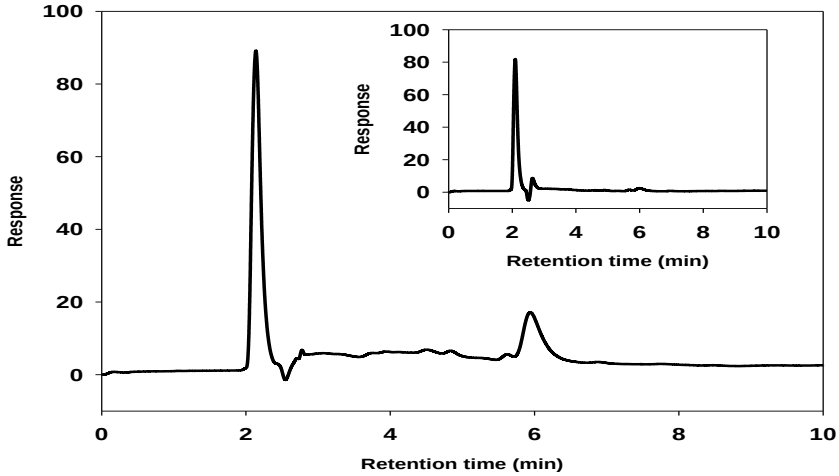

Fig. 4: Demonstration of the hydrolytic cleavage of the ester bond of cypermethrin by *Psychrobacter faecalis* strain by HPLC analysis of cell-free culture supernatant after 10 days of growth. The arrow indicates the accumulation of 3-phenoxybenzoic acid. The insert shows a control with heat-inactivated cells (80 ^{0}C, 30 min).

Bacterial Growth and Biodegradation of Cypermethrin in Mineral Salts Medium

The ability of *Psychrobacter faecalis* AB2 to grow at the expense of cypermethrin (doubling time about 27.79 h and growth rate of 0.025 h^{-1}) as the sole source of energy and carbon showed high utilization of cypermethrin within 72 h (Fig. 5). Akbar et al. (2015a) reported that three bacterial strains (*Ochrobactrum anthropi* JCm1, *Bacillus megaterium* JCm2, and *Rhodococcus* sp. JCm5) degraded 86 – 100% of cypermethrin (100 mg L^{-1}) within 10 days of incubation. Guo et al. (2019) isolated *Pseudomonas* sp. capable of degrading 60 mg L^{-1} to 6 mg L^{-1} of cypermethrin in 20 days, which is similar to the results obtained from this study. The population of *Psychrobacter faecalis* strain AB2 increased from 1.7 x 10^7 to 9.2 x 10^8 cell count ml^{-1} between 0 and 3 days during growth on cypermethrin as sole source of carbon and energy (Fig. 5). This was followed by a decline to 6.4 x 10^8 cell count ml^{-1} by day 4 before it increased again to 9.9 x 10^8 cell count ml^{-1} on day 10. The decrease in the population count of strain AB2 at day 4 was due to the release of cypermethrin metabolites in the culture medium, which may be toxic to the bacterial strain. Growth of the bacterium expressed as the increase in optical density was tightly correlated with the increase in cell count in the culture medium. Heat-inactivated cells (incubated for 30 min at 80 ^{o}C) used as controls showed no increase in biomass, and this further verifies that the increase in biomass was due to the utilization of cypermethrin as a carbon and energy source.

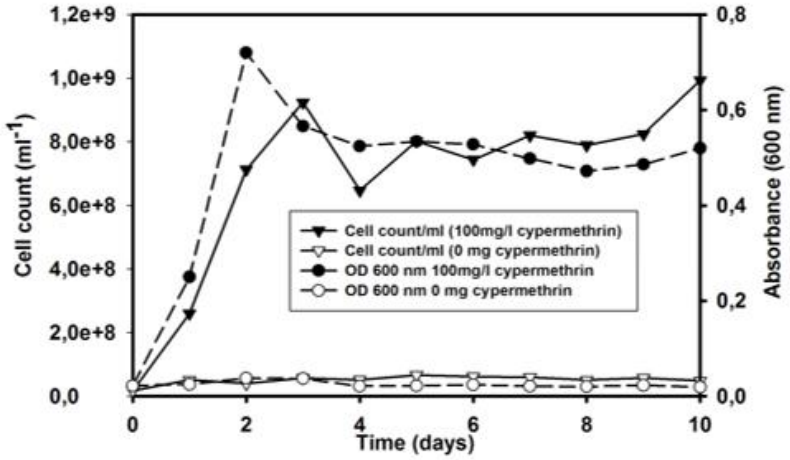

Fig. 5: Growth of *Psychrobacter faecalis* strain AB2 with cypermethrin as sole source of carbon and energy

GC analysis of the residual concentration of cypermethrin showed that *Psychrobacter faecalis* strain AB2 utilized 74.19 % of cypermethrin in MSM with a degradation rate of 0.61 mg L^{-1} d^{-1} (Fig. 6). Control flasks inoculated with heat-inactivated cells (80 ^{o}C, 30 min) of *Psychrobacter faecalis* strain AB2 showed an insignificant decrease in residual cypermethrin concentration (6.90 %) after 10 days of incubation. This decrease could be a result of abiotic loss. Analysis of culture supernatants from cypermethrin mineral salts medium via GC-FID revealed significant reductions in the residual concentration of cypermethrin over time during the growth

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experiment. The percentage residual cypermethrin degradation in pure cultures of strain AB2 was 25.81 %, indicating rapid utilization of the substrate by the organism (Fig. 5). Three bacterial strains isolated from agricultural soil and identified as *Acinetobacter calcoacetius* MCm5, *Brevibacillus parabrevis* FCm9, and *Sphingomonas* sp. RCm6 were found highly efficient in degrading 100 mg L^{-1} of cypermethrin and other pyrethroids after 10 days of incubation (Akbar et al., 2015b).

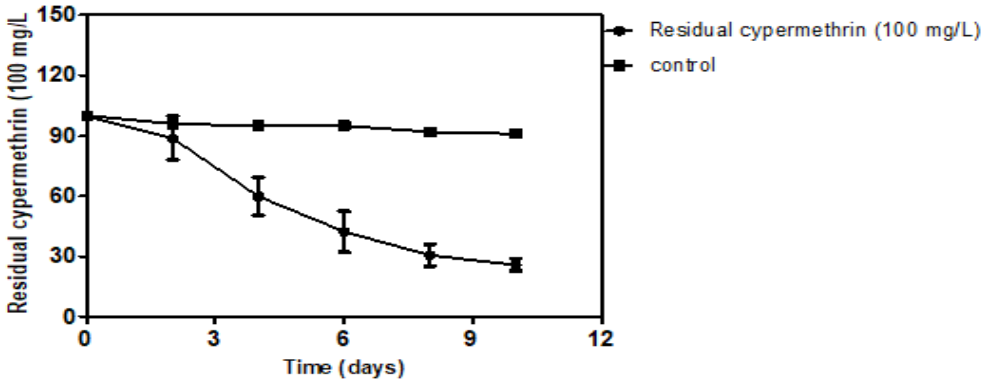

Fig. 6: Utilization of cypermethrin (100 mg L^{-1}) by *Psychrobacter faecalis* strain AB2 as sole source of carbon and energy.

Microcosm experiment

Residual cypermethrin present in soil microcosms, as detected by gas chromatography are shown in Fig. 7. The biodegradation efficiency of *Psychrobacter faecalis* AB2 was estimated through a decrease in the residual cypermethrin concentration under controlled conditions. In a soil bioremediation study, there was a significant difference ($p < 0.05$) in cypermethrin degradation rate between non-inoculated sterilized soil samples (Ni-SS) and soil samples inoculated with *Psychrobacter faecalis* strain AB2 (SS and NSS). The percentage cypermethrin degradation was 40.58 % and 37.23 % in non-sterilized soil (NSS) and sterilized soil (SS) inoculated with strain AB2, while 22.91 % was recorded in non-inoculated sterilized soil samples after 10 days of incubation. Soil bioremediation studies showed that cypermethrin (100 mg kg^{-1}) was rapidly utilized in non-sterilized soils inoculated with *Psychrobacter faecalis* strain AB2 when compared with non-inoculated sterilized soil. Non-sterilized soil inoculated with *Psychrobacter faecalis* strain AB2 recorded the highest (60.19 %) degradation of cypermethrin compared to the control treatment (Ni-SS) after 20 days of incubation. This indicates that cypermethrin degradation was enhanced by the isolated microorganism and also the indigenous microorganisms present in the soil samples during the 20 days. The pesticide residues remaining in the soils indicated incomplete degradation of cypermethrin after 20 days of the experiment. This result was in agreement with the findings of other studies. For instance, Chen et al. (2011) observed that the addition of *Stenotrophomonas* sp. strain ZS-S-01 in soils could significantly enhance the degradation of synthetic pyrethroids and their metabolites. Also, Akbar et al. (2015a) recorded that in sterilized non-inoculated soil, cypermethrin disappearance was insignificant, and 90 % of applied cypermethrin persisted at the end of 42 days of incubation, while 100 % of cypermethrin was degraded within the same period by *Rhodococcus* sp. JCm5. This is an important feature of an organism to be employed for the bioremediation of the natural environment. These findings indicated that the inoculated strain could co-metabolize the pollutants with the indigenous microorganisms.

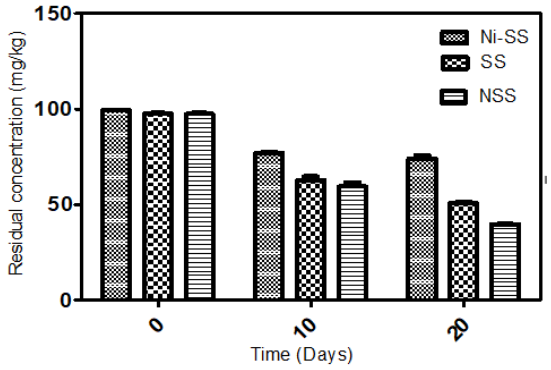

Fig. 7: Degradation of cypermethrin (mg kg^{-1}) in agricultural soil treated with cypermethrin after 20 days of incubation. Ni-SS = Non-inoculated sterilized soil; CYP = Cypermethrin, SS = Sterilized soil, cypermethrin and strain AB2, and NSS = Non-sterilized soil, cypermethrin and strain AB2.

Cross-Feeding Studies

The ability of the isolate to utilize a range of aromatic compounds as carbon sources is shown in Table 1. *Psychrobacter faecalis* strain AB2 was able to utilize 4-chlorobenzoic acid, 3-hydroxybenzoic acid, and 4-bromobenzoic acid as carbon and energy sources. Other aromatic compounds utilized by strain AB2 include benzoic acid, salicylic acid, and catechol. However, strain AB2 was unable to grow at the expense of 3-PBA as a carbon source (data not shown). This might be attributed to its antimicrobial activities (Pepi et al., 2011). The utilization of a wide range of aromatic compounds by *Psychrobacter faecalis* strain AB2 enhances its potential in the bioremediation of a polluted environment.

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Table 1: Cross-feeding studies of *Psychrobacter faecalis* strain AB2

Substrate	Strain AB2	OD600
2,4,5-Trichlorobenzoic acid	+	0.4
3,4-Dichlorobenzoic acid	+	0.3
2,4-Dichlorobenzoic acid	+	0.3
3-Chlorobenzoic acid	+	0.5
4-Chlorobenzoic acid	+	0.4
2,5-Dihydroxybenzoic acid	+	0.3
3-Hydroxybenzoic acid	+	0.4
4-Bromobenzoic acid	+	0.5
2-Bromobenzoic acid	+	0.4
2-Fluorobenzoic acid	+	0.3
4-Fluorobenzoic acid	+	0.4
3-Phenoxybenzoic acid	-	0.0
Salicylic acid	+	0.5
Benzoic acid	+	0.7
Phenol	+	0.5
Catechol	+	2.0

+ = Growth detected via an increase in optical density at 600 nm in mineral salts medium after 30 days of incubation at 28 °C in comparison to a control without any carbon source
- = No detectable growth in mineral salts via increase in optical density at 600 nm in mineral salts medium after 30 days of incubation at 28 °C

Based on the results obtained from HPLC measurements and cross-feeding studies, a pathway for the degradation of cypermethrin and 3-PBA by *Psychrobacter faecalis* strain AB2 was constructed (Fig. 8).

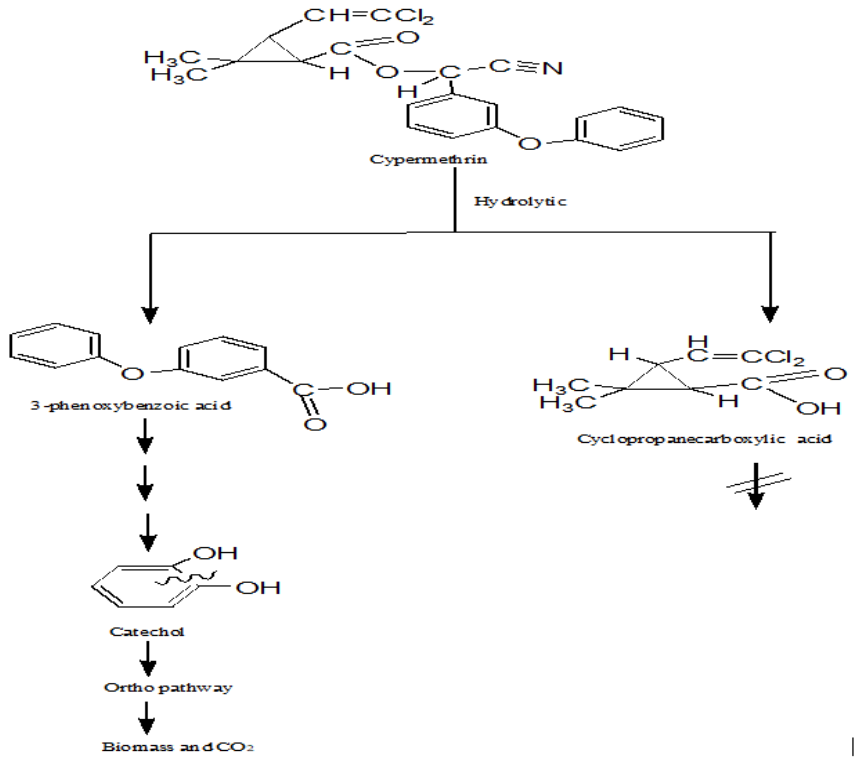

Fig. 8: Proposed pathway for the aerobic biodegradation of cypermethrin and 3-PBA by *Psychrobacter faecalis* strain AB2 based on the detected metabolites

Conclusion

The isolation and characterization of a cypermethrin catabolizing strain of *Psychrobacter faecalis* AB2 from a tropical environment shows the ecophysiological versatility and biodiversity of members of this genus. It points to their possible contribution and use in the bioremediation of aromatic compounds. *Psychrobacter faecalis* strain AB2 was able to utilize cypermethrin (74.19 % of cypermethrin in MSM with a degradation rate of 0.61 mg L^{-1} d^{-1}) as the sole source of carbon and energy after 10 days of incubation. The bacterial strain was able to utilize a range of other aromatic compounds with OD_{600} values from 0.2 to 0.7 after 30 days of incubation. However, it was unable to use the 3-phenoxybenzoic acid metabolite accumulated in the culture medium. This indicates that the activity of other microorganisms is required to mineralize 3-phenoxybenzoic acid due to its antimicrobial activity in the ecosystem.

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The authors declare that they have no conflict of interest.

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