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**REF: UU/AC/ROI/VOL.3/24** 6th November 2018

**-CHRISTABEL MUHONJA--**
**-MACHAKOS UNIVERSITY--**
**-P. O BOX 136-90100-**
**-MACHAKOS---**

Dear Sir/Madam,

**RE: INVITATION TO AN INTERNATIONAL CONFERENCE ON SCIENCE, TECHNOLOGY AND INNOVATION FOR SUSTAINABLE DEVELOPMENT IN DRYLAND ENVIRONMENTS**

Umma University in Kajiado County, South Eastern Kenya University (SEKU) in Kitui County, Lukenya University in Makueni County and Machakos University in Machakos County, together with other partners, are jointly organizing an international Conference entitled *"Science, Technology and Innovation for Sustainable Development in Dryland Environments"* to be held on 19th-23rd November 2018. The theme of the conference is *"Harnessing Dryland Natural Resources for Sustainable Livelihoods in the Era of Climate Change"*. The conference will be two-phased with a two day pre-conference training workshop on 19th-20th November 2018 at SEKU and the main conference on 21st-23rd November 2018 at Umma University. The conference will provide an excellent platform for the academia from around the world to engage with the industry, innovators, policy makers, value chain developers, farmers, and service providers among others so that higher education in Africa contributes to solving the problems of natural resources governance in the era of climate change.

We are therefore pleased to invite you to attend the pre-conference training workshop at SEKU Main Campus in Kitui on 19th-20th November 2018 and the Main conference at Umma University on 21st to 23rd November 2018. Please note that you will be responsible for your travel and accommodation arrangements and conference registration fee.

Yours Sincerely,

**DR. ALI ADAN ALI**
**FOR THE: VICE-CHANCELLOR**

# (CONFERENCE 2018 ABSTRACT)

Inbox x

**Ali Adan <aadan@umma.ac.ke>** Oct 15, 2018, 12:54 PM (3 days ago)

to: me, ird@umma.ac.ke

Dear Dr. Christabel,

Good Afternoon,

I hope and pray that you are doing well.

On behalf of conference organizing committee, I have the pleasure to inform you that your abstract titled: **Molecular and Biochemical Characterization of Poly-Ethene (PE) degrading bacteria and fungi from Dandora dumpsite Nairobi-Kenya** has been accepted for conference presentation. You are kindly requested to prepare full paper for the preparation of the conference proceedings and register and also pay for the preconference and conference. Both is a single charge. The Full paper to reach the conference secretariat by close of business 10th November 2018.

May God Almighty bless you all. Congratulation.

Dr. Ali Adan Ali, PhD; MSc.; MEIK, FASI; NEMA Lead Expert
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# Molecular Characterization of Low Density Poly-ethene (LDPE) degrading bacteria and fungi from Dandora dumpsite Nairobi-Kenya

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## ABSTRACT

This study aimed at molecular and biochemical characterization of Low Density Poly-ethene (LDPE) degrading fungi and bacteria from Dandora dumpsite-Nairobi. Twenty bacterial and 10 fungal isolates were identified using 16S rDNA and 18S rDNA sequences for bacteria and fungi respectively. The highest fungal degradation was attributed to *Aspergillus oryzae* strain A5, 1 while the highest bacterial degradation was attributed to *Bacillus cereus* strain A5,a and *Brevibacillus borstelensis* strain B2,2 respectively. Isolates were screened for their ability to produce extra cellular laccase and esterase; *Aspergillus fumigatus* strain B2,2 exhibited the highest presence laccase (15.67mm) while *Aspergillus oryzae* strain A5,1 exhibited the highest presence of esterase (14.33 mm). Alkane hydroxylase encoding genes were screened for using primer AlkB 1 which amplified the fragment of size 870 bp. Four bacterial samples were positive for the gene. Optimum growth temperature of the fungal isolates was 30°C. The possession of laccase, esterase and alkane hydroxylase activities are suggested as key molecular basis for LDPE degrading capacity. Knowledge of optimum growth conditions will serve to better utilize microbes in the bioremediation of LDPE. The application of *Aspergillus oryzae* strain A5, 1 and *Bacillus cereus* strain A5,a in poly-ethene degradation is a promising option in this kind of bioremediation as they exhibited significantly high levels of biodegradation. Further investigation of more alkane degrading genes in biodegrading microbes will inform the choice of the right microbial consortia for bioaugmentation strategies.



**Key Words**: Low Density Poly-ethene, *Aspergillus oryzae, Bacillus cereus, Brevibacillus borstelensis, Aspergillus fumigatus,* laccase, esterase, hydroxylase, bioremediation

## 1.0: INTRODUCTION

Low density Poly-ethene is a major cause of environmental pollution due to its high tensile strength, lightness, resistance to water and microbial attack. The consumption of plastics in the country has increased to 4,000 tons per annum of Poly-ethene bags which together with hard plastics end up scattered in the environment creating "the plastics menace" (1). Through the National Environmental Management Authority (NEMA), Kenya has embraced the 3Rs, Reduce, Re-use and Recycle concept of solid waste management (2) and most recently the ban on the use of Poly-ethene carrier bags but this has not addressed the problem of Poly-ethene which remain scattered in the environment (3).

Biodegradation is the decomposition of substances through microbial activity and is a complex process which involves the following steps (4): bio-deterioration, depolymerization, assimilation and mineralization. Bacteria and fungi of various genera have been implicated previously in the biodegradation of Poly-ethene albeit the low rates. *Acinetobacter sp* was found capable of utilizing n-alkanes of chain length C10–C40 as a sole source of carbon as reported by (5). Bacterial genera, namely, *Pseudomonas, Acinetobacter, Brevibacillus, Rhodococcus and Micrococcus* (6, 7 , 1) respectively isolated from different sources proved to be the potential organisms for Poly-ethene degradation. Fungal genera, *Gliocladium, Cunninghamella, Penicillium, Aspergillus, Fusarium, Mucor* and *Mortierella* from soil (1) were proven to have the potential to degrade Poly-ethene after analysis of degradation through various methods.

Plastic bio-degradation as a result of the activity of certain enzymes causes cleavage of the polymer chains into monomers and oligomers. Enzymatically broken down plastic is further absorbed by the microbial cell to be metabolized. Aerobic breakdown produces carbon dioxide and water. The involvement of enzymes in microbial biodegradation of Poly-ethene has been investigated and enzymes like laccases and esterases have been confirmed to play a role in this process either directly or indirectly (8). The production of enzyme laccase in the presence of Poly-ethene as the sole carbon source is a clear indication that laccase has a role in breaking down some of the intermediary products produced during this process. In this study, molecular characterization of bacteria and fungi that had been confirmed to degrade Poly-ethene was done as well as assessment of optimum pH, temperature and sodium chloride concentration at which they can thrive. Presence of Alk B genes that encode alkane hydroxylases known to hydrolyze alkanes was also investigated.

## 2.0: MATERIALS AND METHODS

### 2.1: Bacterial DNA extraction

Total genomic DNA was isolated from the bacterial pure cultures grown to the late exponential phase by means of a standard protocol (9) as follows: 1.5 ml of the overnight bacterial culture (grown in LB medium) was transferred to a 1.5 ml eppendorf tube and centrifuged at 13000 rpm for 1min to pellet the cells. The supernatant was discarded. The cell pellet was suspended in 600 µl TE buffer and centrifuged at 13000rpm and the supernatant discarded. The cell pellet was re-suspended in 200 µl TE buffer and the following were added: 5 µl lysozyme (20mg/ml), 5 µl RNAnase (20mg/ml), 10 µl proteinase K (20mg/ml) followed by overnight incubation at 37°C. The next morning the temperature was adjusted to 56°C for one hr and an equal volume of phenol/chloroform (1:1) was added and mixed well by inverting the tube until the phases were completely mixed. Spinning was done at 13000rpm for 15 min at room temperature. The upper aqueous phase was carefully transferred to a new tube by using 1 ml pipette. This step was repeated twice to ensure all protein had been removed. An equal volume of chloroform isoamyl (24:1) was added to the aqueous layer and centrifuged at 13000rpm for 15 min. The aqueous layer was removed into a new tube. This step was also repeated to ensure all phenol is removed. An equal volume of isopropanol was added and stored overnight at -20 °C. The samples were then defrosted and centrifuged at 4°C for 30 min to pellet the DNA. The pellet was washed in 70% ethanol and centrifuged at 13000rpm for 5 min then the ethanol was carefully pipetted out. The pellet was air dried on the bench for 20 min and the isolated genomic DNA was viewed on a 1% agarose gel.

### 2.2: Bacterial DNA amplification and sequencing

Amplification of the 5' end of the 16S rDNA gene was performed with universal primers: forward primer (8-F)5'-AGAGTTTGATYMTGGCTCAG-3' and reverse primer: (1942R)5'-GGTTACCTTGTTACGACTT-3' (10). The PCR was performed on a GeneAmp PCR system 9600 (Applied Biosystems), using 1 µl Taq Polymerase (Applied Biosystems), 1 µl each of 10 pM concentrations of forward and reverse primers, 27 µl sterile deionized water, 8 µl PCR buffer containing dNTPs and $MgCl_2$, and 2 µl DNA template, for a total reaction volume of 40 µl. The cycling program used was as follows: 1 cycle of 94°C for 5 min; 30 cycles of 94°Cfor 30 sec, 55°C for 30 sec, and 72°C for 1.5 min; and a final extension of 72°C for 10 min. The PCR products were visualized through electrophoresis on a 1% agarose gel with ethidium bromide added directly. The 1.5 kbp products were subjected to Sanger di-deoxy sequencing using the forward primer and reverse primers at Macrogen DNA, Inc. (Netherlands). Sequence files were edited using Chromas version 2.6.2 and compared to the GenBank nucleotide database using

the Basic Local Alignment Search Tool (BLAST). Phylogenetic relationships were inferred using Mega 7 (11) and maximum-likelihood algorithms available in Phylip. Maximum likelihood and parsimony-derived trees were bootstrapped using PHYML (12, 13)

**2.3: Fungal DNA extraction**

Fungal DNA extraction protocol by (14) was used. Fungal mycelia were grown for 7days at 55°C on potato dextrose agar. Mycelia was frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. The powder was transferred into 2ml tubes and 600µl of preheated extraction buffer added. The contents were incubated in a water bath at 65 °C for 30 minutes with mixing after every 10 minutes. 270µl volume of 5M potassium acetate was added and centrifuged at 13000rpm for 10 minutes. 700µL of the supernatant was transferred into clean tubes volume and 5µL RNASE (10mg) added then incubated for 30 minutes at 37°C. Chloroform and iso-amyl alcohol was prepared in the ratio of 24:1 and an equal volume added to the mixture. 600µL of supernatant was pipetted into clean tubes. DNA was precipitated the by adding a tenth of the volume of 3M potassium acetate and two thirds of the volume of isopropanol. This was incubated at -20°C for 30 minutes then centrifuged at 13000rpm for 10 minutes. The pellet was washed using 70% ethanol followed by 10 minutes of centrifuging then the DNA was eluted in 50µL of RNASE-free water and stored at -20°C.

**2.4: Fungal DNA amplification and sequencing**

PCR was performed on a GeneAmp PCR system 9600 (Applied Biosystems), using 1 µl Taq Polymerase (Applied Biosystems), 1 µl each of 10 pM of forward and reverse primers, 27 µl deionized water, 8 µl PCR buffer containing dNTPs and $MgCl_2$, and 2 µl DNA template, for a total reaction volume of 40 µl. The cycling program used were as follows: 1 cycle of 95°C for 5 min; 35 cycles of 95°Cfor 30 sec, 60°C for 45 sec, and 72°C for 40 sec; and a final extension of 72°C for 5 min. Primer pair **F-**566:5' - CAGCAGCCGCGGTAATTCC - 3' and for R- 1200:5'- CCCGTGTTG AGTCAAATTAAGC - 3' which amplify on average a 650 bp long fragment from the V4 and V5 regions were used (15). The PCR products were visualized through electrophoresis on a 1% agarose gel with ethidium bromide added directly. The products were subjected to Sanger dideoxy sequencing by Macrogen, Inc. (Netherlands). SeqMan Pro was used to assemble both the forward and reverse sequence file (16) .The sequences obtained were compared against the sequences available in the NCBI, database using the basic local alignment tool (BLASTn). The 18S rDNA gene sequences obtained in current study, together with those of the closest neighbor strains were aligned using ClustaX version 2.1. Phylogenetic relationships were inferred using Mega 7 (11) and maximum-likelihood algorithms available in Phylip. Maximum likelihood and parsimony-derived trees were bootstrapped using PHYML (12,13 ).

### 2.5.: Screening for production of enzymes

Bacterial isolates were screened for their ability to produce extracellular enzymes i.e. laccases and esterases. The ability of the isolates to utilize substrates such as lignin and tween 20 exhibited their ability to produce the respective enzymes (17).

### 2.5.1: Determination of presence of enzyme laccase

The media for selection of lignin modifying fungi was prepared by the use of plain agar and minimal salt media with the incorporation of lignin (to encourage selection of lygninolytic fungi) and Guaiacol, which acts as a colorimetric indicator of the lignin-modifying enzymes laccase or peroxidases. All chemicals were obtained from Sigma Chemical Co., St. Louis. The presence of a reddish coloration after 3-5 days of incubation was an indication of laccase activity. The Laccase assay per 1 liter: 400 µl Guaiacol, agar 15g, 2 g Malt extract, 0.5 g $KH_2PO_4$, 0.001 g $ZnSO_4$, 0.4 g $K_2HPO_4$, 0.02 g $FeSO_4$, and 0.2 g $MgSO_4$, 0.5g $KH_2PO_4$, 0.1g $NH_4NO_3$, 0.1g KCl, 5ml KOH, 0.25g Chloramphenicol, forming a reddish colored zone as a positive result.

### 2.5.2: Determination of presence of enzyme esterase

The isolates were cultured on basal media (1 % $KH_2PO_4$, 0.0 1% $MgSO_4.7H_2O$, 0.005 % $CaCl_2.2H_2O$, 4 % NaCl and 1 % $Na_2CO_3$) supplemented with 1 % tween 20 (domestic grade) as the sole carbon source. The medium was then thereafter inoculated by the spotting of isolates per plate and incubated for at least 48 hours at 37°C for bacteria and at 28°C for 3-5 days for fungal isolates. The media was observed for zones of precipitation of calcium crystals around each isolate. Positive isolates for esterases production were indicated by the precipitation of calcium crystals around the colonies.

### 2.5.3: Screening for genes producing alkane degrading enzymes

Amplification was done using the sets of Alk B primers (18) shown in **Table 1**. The PCR was performed on a GeneAmp PCR system 9600 (Applied Biosystems) using Taq DNA polymerase. A total of 30 cycles of amplification was performed with template DNA denaturation at 94 °C for 1 min, primer annealing at 40 °C for 1 min and primer extension at 72 °C for 2 min (19). The PCR products were visualized through electrophoresis on a 1% agarose gel with 2µl ethidium bromide added directly.

**Table 1: Primers for Alk B genes encoding depolymerases responsible for alkane degradation** (18)

| Primers and position | PCR product | Reference |
|---|---|---|
| ***alkB 1* set 1** | | |
| 82 5'- TGGCCGGCTACTCCGATGATCGGAATCTGG-3' 111 | 870 bp | Kok et al. |
| 951 5'- CGCGTGGTGATCCGAGTGCCGCTGAAGGTG-3' 922 | | |
| ***alkB1** set 2* | | |
| 134 5'- CATTTCCCTGGTGATTG-3' 151 | 718 bp | Stover et al |
| 851 5'- CCGTCCTCGCCCTTTCGC-3' 834 | | |
| ***alkB2*** | | |
| 134 5'- CCTGGCTGGTGATCAGCG-3' 151 | 749 bp | Stover et al. |
| 882 5'- CGAGTGTTCCGGCGTGGTG-3' 864 | | |

### 2.6: Effect of temperature on growth of fungal isolates

Potato Dextrose Agar augmented with 250mg/ml ampicillin to inhibit bacterial growth at pH 7.0 was prepared, sterilized and dispensed in sterile petri dishes. Each plate was inoculated with one fungal isolate and incubated at temperatures 20, 30, and 40°C. Growth of isolates was checked after 4 days of incubation. The level of growth was scored using the colony diameter, whereby (0 mm) indicated no growth, (1-2 mm) indicated minimal growth, (3-4 mm) indicated average growth (5-7 mm) indicated satisfactory growth while (8-10 mm) indicated excellent growth.

## 3.0: RESULTS AND DISCUSSION

### 3.1: Phylogenetic relatedness of low density poly-ethene degrading fungal isolates

Amplification of fungal 18S rDNA using 1200R and 566F universal primers yielded the expected band size of approximately 640 bps from the PCR products of the amplified samples (**Fig 1**). These products were purified, sequenced and analyzed. The results were used to obtain accession numbers from NCBI GenBank. The analyzed sequences were aligned with those of the

closest neighbors using ClustalX version 2.1. Phylogenetic relationships were inferred from phylogenetic comparison of the 18S rDNA sequences using Mega 7 and maximum-likelihood algorithms to generate the phylogenetic tree (**Fig 2**) which shows the phylogenetic relationships among the various *Aspergillus* species. The tree displays four clades in which the isolates have been clustered. From our previous study (20), Aspergillus oryzae (MG779508) resulted in a weight loss of 36.4±5.53% which was the highest. Aspergillus oryzae is a promising biodegrader of poly-ethene as it was able to degrade 30% of poly-ethene in 200 days (21) in addition to formation of micro cracks and increased embrittlement of the LDPE surface upon SEM analysis. In a study done using untreated LDPE incubated with A. oryzae, 5% weight loss was recorded compared with control (untreated and unexposed). Aspergillus fumigatus strain B2,2 (MG779513) recorded a weight reduction of 24±3.26% which was the second highest in our previous study (20). *Aspergillus fumigatus* is also among the species that have been investigated for their ability to degrade poly-ethene and other polymers. In a study, three fungal species were investigated for their ability to degrade poly-ethene and *A. fumigatus* was the best degrader compared to *A. terreus* and *F. solani* following an analysis of the LDPE surface by SEM and FTIR (22). Other fungi implicated in this study included *Aspergillus nidulans*, *A flavus, A. terreus* and *A. neoflavipes* which resulted in weight loss of the LDPE sheets. Use of weight reduction as a measure of the extent of poly-ethene biodegradation has been widely accepted and used by many authors (23).

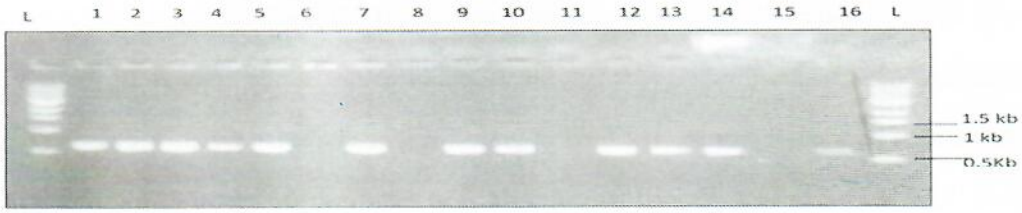


**Fig 1:** PCR products for the amplification of 18S r DNA for the fungal isolates 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 using 1200R and 566F universal primers. L represents a 1 kb ladder. The expected band size amplified is 640 bps.

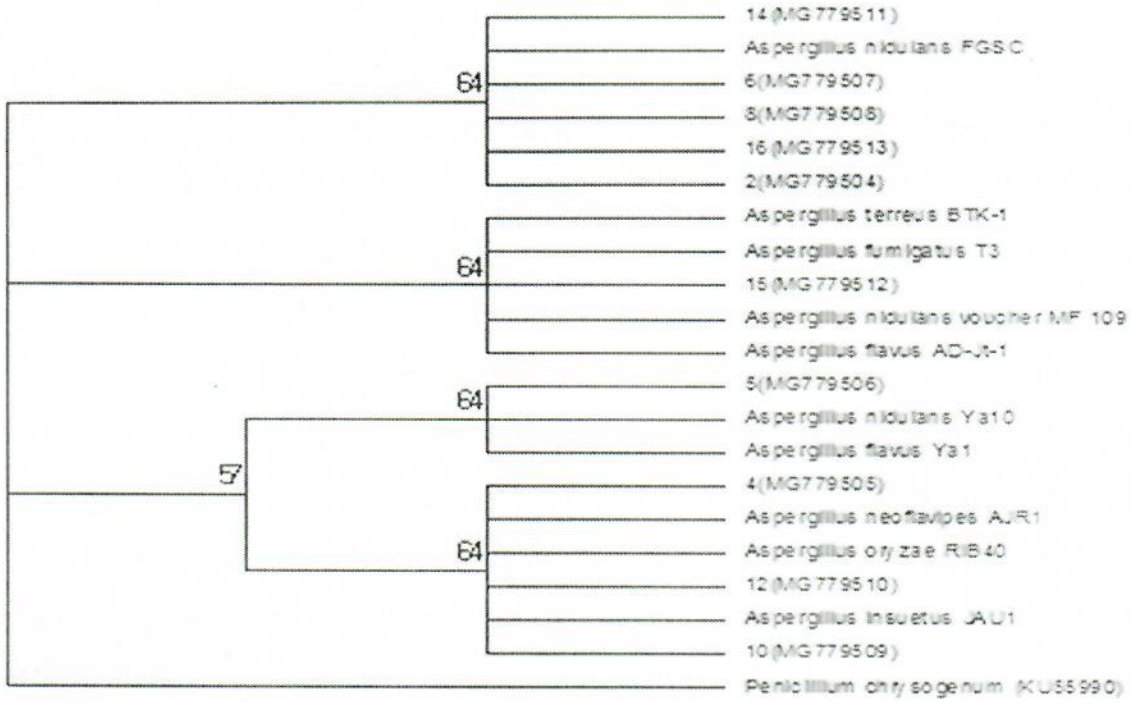


**Fig 2:** Phylogenetic tree of fungal isolates based on 18S rDNA sequences. All screened fungal isolates have NCBI accession codes in brackets. The scale bar refers to 0.007 substitutions per nucleotide position. Bootstrap values obtained with 1000 resampling are referred to as percentages at all branches.

### 3.2: Phylogenetic relatedness of low density poly-ethene degrading bacterial isolates

Amplification of bacterial 16S r DNA using 1492R and 8F universal primers yielded 1420 bps fragments **(Fig 3)** which were purified, sequenced and analyzed. The results were used to obtain accession numbers from NCBI GenBank. The analyzed sequences were aligned with those of the closest neighbors using ClustaX version 2.1. Phylogenetic relationships were inferred from phylogenetic comparison of the 16S rDNA sequences using Mega 7 and maximum-likelihood algorithms to generate the phylogenetic tree (**Fig 4**) which shows the phylogenetic relationships among the genera and species. *Brevibacillus, Bacillus* and *Lysinibacillus* are in one major clade while *Pseudomonas, Ochrobactrum* and *Cellulosimicrobium* are in grouped in another major clade. Bacteria of the genera *Bacillus, Brevibacillus, Ochrobactrum, Lysinibacillus, Cellulosimicrobium* and *Pseudomonas* were identified as effective poly-ethene degraders. Bacterial isolates A5, 1a-*Bacillus cereus* (MG645256) produced the highest degradation effectiveness in terms of weight loss i.e. 35.2% followed by isolate B2,2-*Brevibacillus borstelensis* (MG645267)-20.28% from an earlier study (20) while isolates B1,1a *Pseudomonas putida* (MG645283)-2.88% and D4,yn-*Brevibacillus borstelensis* strain (MG645261)- 6.8%. The genus *Bacillus* was the most frequently identified among the LDPE biodegrading genera in this study. Species identified under this genus include *Bacillus cereus, Bacillus toyonensis, Bacillus thuringiensis, Bacillus subtilis, Bacillus pseudomycoides, Bacillus safensis* and *Bacillus niacini.* Various studies have been done to investigate

the efficacy of genus *Bacillus* in poly-ethene degradation and different species under this genus have been found to have potential to degrade poly-ethene (24, 25). *Bacillus cereus* has been found to be a good bioremediation candidate in the biodegradation of poly-ethene due to its ability to produce enzymes laccase and manganese peroxidase. In a comparative study, *B. cereus* was found to be more effective than *B. sphericus* in degrading photo-oxidized and thermos-oxidized LDPE (26). According to (6), *Brevibacillus borstelensis*-Accession number- AY764129 was able to degrade 11% of nonirradiated poly-ethene by weight in 30 days . Two bacterial isolates *Bacillus amyloliquefaciens* (BSM-1) and *Bacillus amyloliquefaciens* (BSM-2) were isolated from municipal soil and used for polymer degradation studies and were found to produce significant changes on LDPE in terms of weight loss, reduction of tensile strength and appearance of new functional groups (27). A novel strain of Pseudomonas; *Pseudomonas citronellolis* EMBS027, GenBank Accession number KF361478 was isolated by (28) from a municipal landfill in Indore, India and it degraded 17.8% of poly-ethene in 4 days. Different species of Pseudomonas were analyzed for their ability to degrade poly-ethene and upon incubation for 120 days *Pseudomonas putida* resulted in a weight loss of 9% (29).

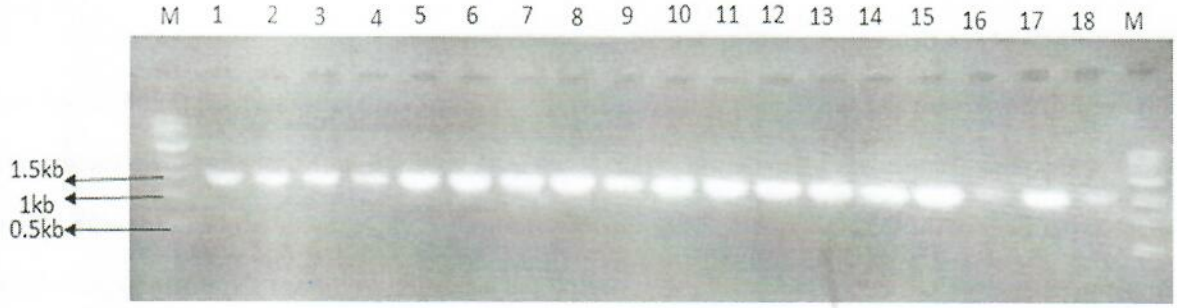


**Fig 3:** Amplification of 16S r DNA for the bacterial isolates 1-18 using 1492R and 8F universal primers. M represents a 1 kb marker. The expected band size amplified is 1420 bps.

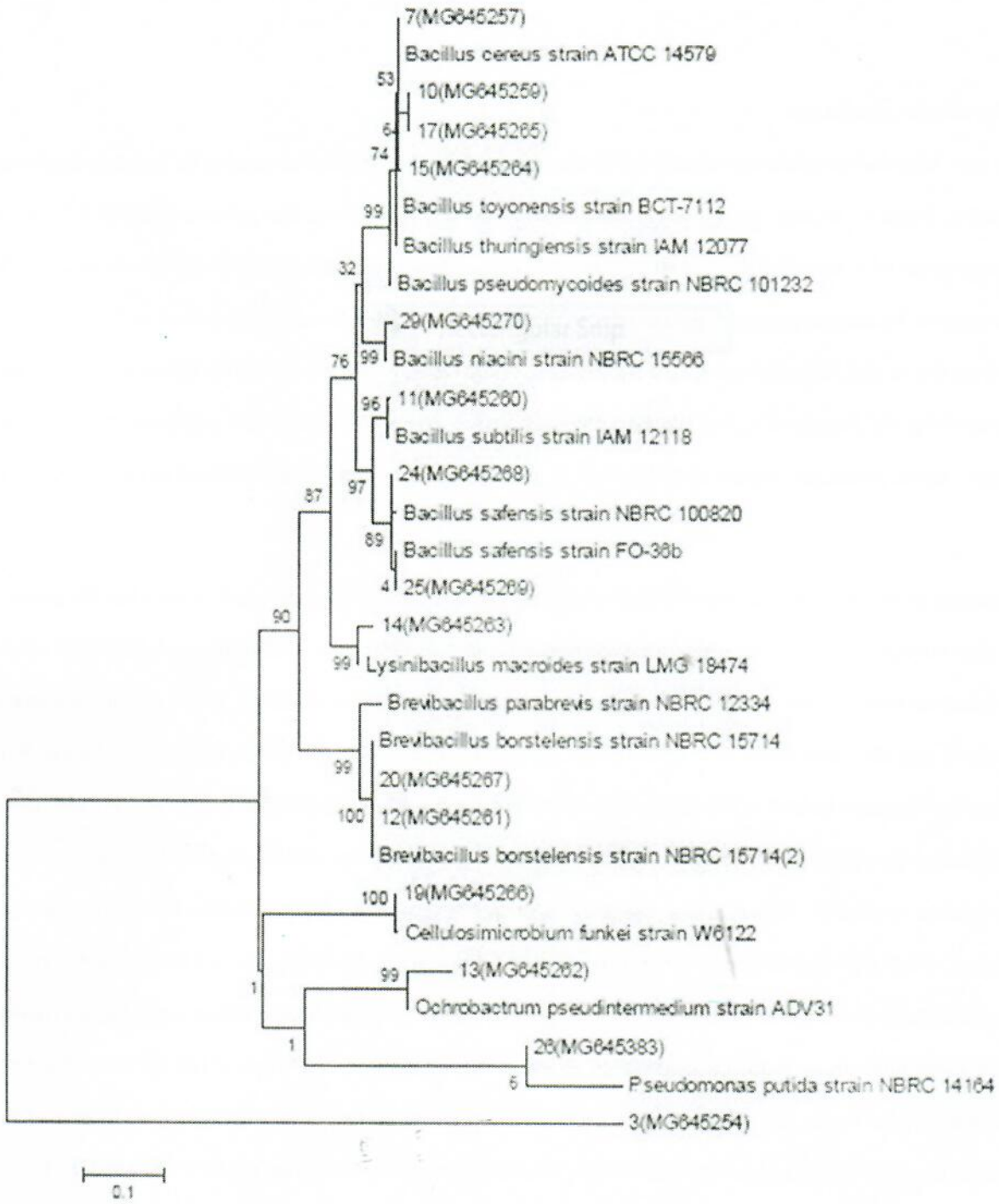


**Fig 4:** Phylogenetic tree generated by MEGA 7 for 16S rDNA sequences of the bacterial isolates found to be effective bacterial degraders. All screened bacterial isolates have NCBI accession codes in brackets. The scale bar refers to 0.02 substitutions per nucleotide position. Bootstrap values obtained with 1000 resampling are referred to as percentages at all branches

### 3.3: Screening for enzyme production

Bacterial isolates were screened for production of enzymes laccase and esterase (**Fig 5**) which are among the enzymes implicated in LDPE degradation. Bacterial isolates *Brevibacillus borstelensis* strain B2, 2, *Brevibacillus parabrevis* strain C2, 2a and *Pseudomonas putida* strain B1,1 exhibited the highest presence of laccase. Only two isolates: *Bacillus toyonensis* and *Bacillus macrolides* were negative for laccase activity. Esterase activity was highest in isolates *Brevibacillus borstelensis* strain D4 yn, *Bacillus niacin* strain C4,1a and *Pseudomonas putida* strain B1,1a. Fungal isolates were screened for production of enzymes laccase and esterase (**Fig 6**). Isolates B2, 2-*Aspergillus fumigatus*, A5,1-*Aspergillus oryzae* and A4,2a-*Aspergillus flavus* exhibited the highest levels of laccase enzyme while the highest level of esterase enzyme was attributed to fungi *Aspergillus Oryzae*.

Production of extracellular enzymes plays an important role in polymer degradation through depolymerization where the polymer is broken down into smaller sub units (30) which are then enzymatically degraded into intermediary products that can be assimilated into microbial cells (31) and utilized as carbon sources leading to production of energy, water, carbon dioxide and methane in the case of anaerobic respiration (32). In this study, production of extracellular enzymes laccase and esterase, were investigated. Fungal and bacterial isolates in this study were scrutinized for their ability to produce laccase enzyme and isolate B2, 2: *Aspergillus fumigatus* (MG779513) which had a LDPE degradation effectiveness of 24%, had the highest diameter of coloration due to laccase production. This could be attributed to its ability to produce higher amounts of laccase and other extracellular enzymes which are believed to play a role in poly-ethene degradation. According to (33), the production of this enzyme increases when the microbes are in close proximity with the poly-ethene. (34) were able to extract crude laccase enzyme which was incubated with poly-ethene and led to degradation as was evidenced through weight loss, FTIR and SEM. Esterases catalyze the cleavage of ester bonds (35) of short-chain triglycerides or esters. Esters have been identified as part of the intermediary products produced during poly-ethene degradation when the post incubation culture media is subjected to GC-MS analysis that can be assimilated into the microbial cells, undergo hydrolysis to give rise to the subsequent carboxylic acid and alcohol that ultimately undergo respiration to produce energy (36). Isolate A5,1- *Aspergillus oryzae (*MG779508) with a weight loss of 36.4% had a high activity of enzyme esterase of 10%. This could have contributed to its high degradation potential compared to other fungal isolates which had lower degradation

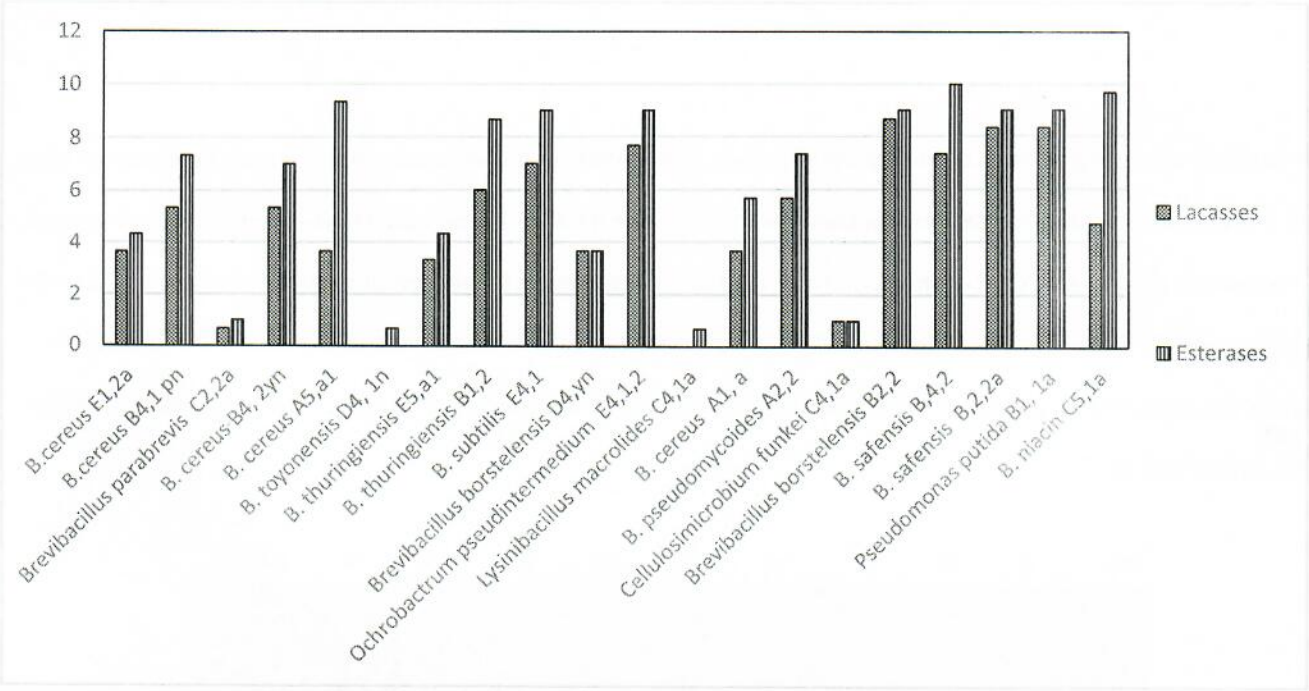


**Fig 5:** Presence of enzymes Laccase and Esterase among the bacterial isolates. Growth of bacterial isolates was measured as colony diameter (mm). Means were grouped using Tukey's Honest Significant Difference test at ($P<0.05$).

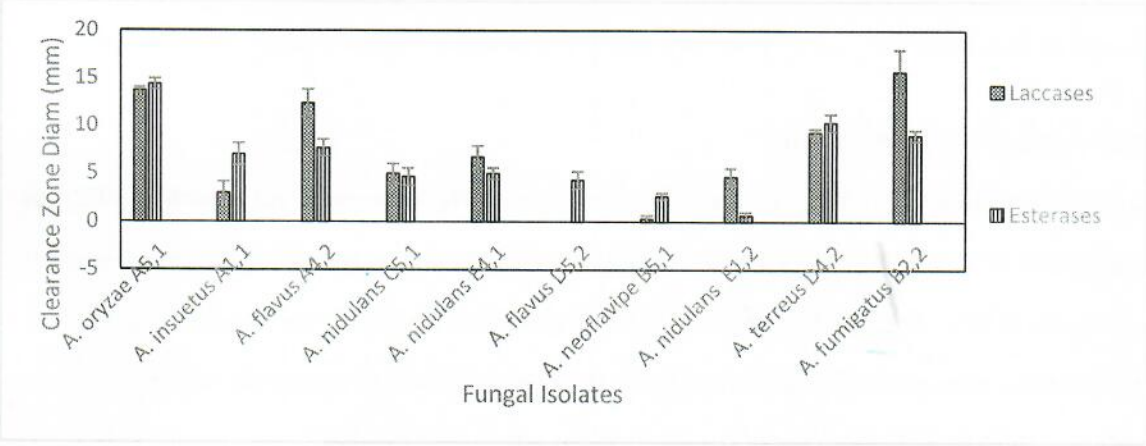


**Fig 6**: Presence of enzymes Laccase and Esterase among the fungal isolates. Growth of fungal isolates was measured as colony diameter (mm). Means were grouped using Tukey's Honest Significant Difference test at ($P<0.05$).

### 3.4: Screening for AlkB genes producing alkane degrading enzymes

PCR to amplify Alk B genes was done using three sets of Alk B primers. Only one set of the Alk B primers was able to amplify the Alk B gene producing a fragment of size 870 bps. Alk B genes are responsible for production hydrolase enzymes which are responsible for alkane degradation. The gene was amplified in 4 bacterial samples. **(Fig 7)**. A common feature of many alkane degraders is that they contain multiple alkane hydroxylases with overlapping substrate ranges (37). A*lkB* and *alkB* related genes

code for an alkane degrading enzyme, alkane hydroxylase (38). The analysis of the bacterial samples revealed presence of AlkB 1 gene in 4 of the bacterial samples. Bacterial isolates that were positive for alkB 1 gene were D4 yn-*Brevibacillus borstelensis*, B1,1-*Pseudomonas putida* and A5,a1- *Bacillus cereus.* Alkane biodegradation is initiated through terminal oxidation to the corresponding primary alcohol, which is further oxidized by dehydrogenases to fatty acids which can enter the TCA cycle (39). This genetic information is an indication of the genetic ability of the microorganisms to degrade long- chain alkanes through production of this enzyme.

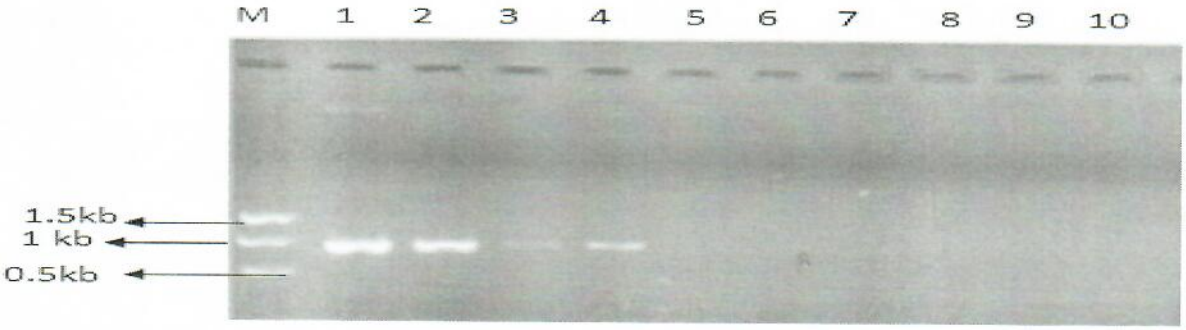


**Fig 7:** PCR products for the amplification of Alk b for the bacterial isolates 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 using Alk B1 set 1 primers. Lane 1 represents a 1 kb ladder. The expected band size amplified is 870 bps.

### 3.5: Effect of temperature on growth of fungal isolates

The growth of fungi at different temperatures (20°C, 30°C and 40°C) as shown **(Fig 8)** revealed that growth at 30°C was significantly higher than growth at 20°C and 40°C with *A. oryzae* strain A5,1 having the highest growth (10±0). However isolate E4, 1-*A. nidulans* grew optimally at 40°C. This could be attributed to the fact that the sampling site for these bacteria was from a dumpsite where the temperatures were generally ambient and hence favoring the growth of mesophilic microbes. Laccase production by fungi is influenced by type and concentration of carbon sources, pH and temperature.

### 3.6: Effect of temperature on growth of fungal isolates

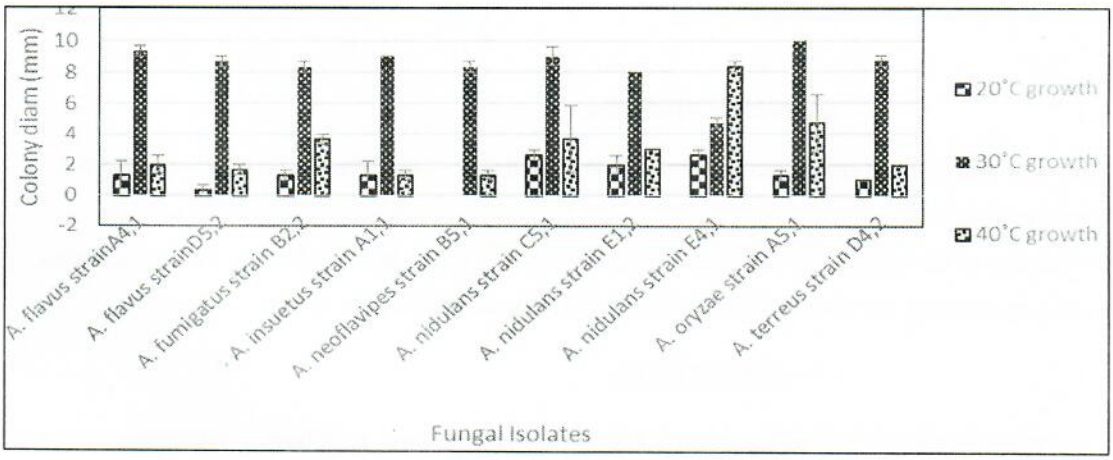


Fig 8: Growth of fungal isolates at 20 °C, 30 °C, and 40°C respectively.

## 4.0: Conclusion

The present work indicates that soil bacteria and fungi isolated from the dumpsite have potential of degrading poly-ethene. This is the first study on the isolation of local bacteria and fungi that can degrade LDPE which is the most common plastic in Kenya. Particularly, the application of *Aspergillus oryzae* strain A5, 1 and *Bacillus cereus* strain A5,a will be beneficial in the bioremediation of poly-ethene as they exhibited significant degradation effectiveness. It was ascertained that the microorganisms are capable of producing enzymes laccase and esterase which have been confirmed to play a role in degradation of poly-ethene. The isolates possess the alkane hydroxylase producing gene (Alk B) which is the molecular explanation for the degradation of LDPE under investigation. Fungi in this study were found to grow optimally at temperature of 30°C.


## Funding Statement

This work has been fully funded by the African Union Commission through the Pan African University Institute of Science, Technology and Innovation.


## Data availability statement

The data used to support the findings of this study are included within the manuscript.

## Conflict of Interest statement

The authors whose names are listed here state that they have NO conflict of interest to declare.

**Acknowledgements**

I highly appreciate the support I got from honest colleagues; Dr. Johnstone Neondo, Dr. Eliud Wafula, Steve Ogada and Baba Ngom who gave me sincere and constructive criticism that helped to shape up this final product. I cannot forget to thank the staff and management of the Institute of Biotechnology Research (IBR); Prof. A.B Nyende, Richard Rotich, Catherine Eyiinda and Grace Mungai for providing an enabling environment for laboratory and research work for this study.

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