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Screening and Optimisation of the Biodegradation Potential for Low Density Polyethylene (LDPE) Films by *Fusarium Equiseti* and *Brevibacillus Parabrevis*

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The accumulation of low density polyethylene, used extensively in packaging for industrial and agricultural applications, in the ecosystem is a great threat. This study focuses on the isolation of micro-biota from the plastic polluted sites to screen and optimise their potential for low density polyethylene (LDPE) film biodegradation. Firstly, the plastic samples from soil dumping plastic debris and plastic polluted water were collected; then fungi and bacteria were isolated using potato dextrose agar media and nutrient agar media, respectively, while screening low density polyethylene film biodegradation performed on mineral salt media (MSM) using the isolated micro-biota. The measurement of the potential biodegradation was assessed by visual observation. The most microbial colonization for low density polyethylene films was identifying molecular which was then utilized for optimisation of the biodegradation processes with different parameters such as media type, inoculum size, shaking speed, different incubation temperature and pH at different incubation time. Then the weight loss in the LDPE films percentage was calculated measuring dry mycelium weight and bacterial absorbance. The results revealed that, among the isolated micro-biota fifteenth, the most colonization was Fusarium equiseti and Brevibacillus parabrevis depending on the scanning electron microsope (SEM) and Fourier transform infrared (FTIR) analysis, in addition to optimum media, inoculum size, shaking speed, incubation temperature, pH, MSM, 2 disks and 2 ml, 30° C and 35°C, pH5 and pH7 for 30:20 days for F.equiseti and B.parabrevis, respectively. The overall results confirmed that F.equiseti and B.parabrevis from the plastic polluted sites play an essential role in low density polyethylene films biodegradation.

Keywords: Biodegradation; Low density polyethylene films; Optimisation; Potential.

Plastic are polymeric compounds, nowadays synthetic plastic apply at many different fields due to their advantages such as durability and tenability[1]. The plastic waste disposal into the environment and land pollution problems has led to concern about plastics waste management [2].

The plastic waste accumulation, caused a serious threat to the environment. Furthermore, swallowing the waste plastic debris by animals affect animal health[3]. Because of the disadvantage of conventional plastic degradation methods, the biological biodegradation methods become more demanding to be apply[4]. Polyethylene is a synthetic plastic commercially produced, while chemically it is petroleum-derived terephthalate acid (TPA) and ethylene glycol. Annually low density polyethylene

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production reached 60%. Due to the absence of appropriate disposal methods, polyethylene wastes are usually burnt in open areas which cause environmental pollution[1].

Biodegradation is eco-friendly process relies on living organisms to degrade the polymeric compounds[5]. There are different factors affecting the plastic biodegradation process such as surface area, organism type, polymer nature, temperature, pH, and the addition of nutrients. The degradation process carried out at different steps. Firstly, the plastic is converted to its simple form, and then are mineralized[6]. Recently, using of microorganisms to degrade the plastic wastes becomes more attractive rather than the chemical and physical disposal methods. The most commonly used methods for the evaluation of plastic biodegradation process are (SEM), and (FTIR) [7]. Moreover, the mass loss of test specimens widely applied degradation tests to measure weight loss or determine residue polymer. The main distinguishing objectives of this study are: screening and optimization the biodegradation potential for low density polyethylene films using *F.equiseti* and *B.parabrevis* as novel microbial strains, and local eco-friendly methods.

MATERIALS AND METHODS

Materials

A. Low density polyethylene (LDPE) used in this study was obtained in the form of LDPE films from plastic bags of market, Cairo, Egypt.

B. Media

Media for microbial isolation were added in g/L. Rose Bengal was added to inhibit bacterial growth; pH was adjusted to pH7 using either (0.1 M) NaOH and (0.1 M) HCL solution, media supplied by Mycology lab, Faculty of science, Helwan University.

1. Potato-Dextrose Agar[8] (PDA)

Infused white potato	200
Glucose	20
Agar	20

2. Nutrient Agar [9]

Peptone	5
Beef extract	3
NaCl	5

Methods

Sample collection

Plastic samples were collected from different plastic polluted sites as shown in Picture (1):

Picture (1): Isolates Sites (www.google.com/maps).

1. Soil dumping of plastic debris was obtained from Tura El-Asmanet Metro Station, Cairo, Egypt as shown in Picture (2E-2F). Biodegradable microorganisms from soil were collected from surface and 10 cm depth. Few grams of soil were transferred into the laboratory and stored until used at room temperature.

2. Water polluted by plastic was obtained from below Abbas Bridge and Scout Club, Cairo, Egypt as shown in Pictures (2A-2D), (3).

Water samples were collected from surface and depth 10 cm through sterile glass bottle (Screw Cap bottles) and stored until used at 4ÚC.

Isolation of micro-biota

Isolation from soil

The collected soil was sieved through mesh with a 2mm pore size sieve; then, two media for fungal and bacterial isolation were used by the isolation method (serial dilution[10]). One gram of soil was added in 9 ml of sterile distilled water, and this suspension vortex was left to settle down.

Next, a series of 10-fold dilution was used to isolate fungi. One ml of each dilution was cultured on potato dextrose agar (PDA); meanwhile, in case of bacteria, nutrient agar media was used.

Isolation from water

Water samples were prepared for fungal and bacterial isolation by serial dilution methods, and the plates were prepared through pouring plate techniques using PDA and Nutrient Agar media. Pure cultures were sub-cultured and then preserved until used.

Screening of the isolated micro-biota biodegradation potential for low density polyethylene films

Basal media were prepared as previously described by Esmaeili et al[11] with a slight modification. Briefly, the synthetic mineral salt media (MSM) composition was as follows: (g/L: K_2HPO_4, 1.2; KH_2PO_4, 0.14; $NaNO_3$, 2; $MgSO_47H_2O$,0.5; KCl, 0.5; $FeSO_47H_2O$, 0.01; $MnSO_4H_2O$, 0.001; $CuSO_45H_2O$, 0.001; $ZnSO_47H_2O$, 0.001; Agar, 15; pH 7.0). Then it was autoclaved and poured into petri-dishes. Suspension of microbial isolates was prepared as described previously by Ibrahim et al[12] by

suspending mycelium plug 8 mm diameter of fungal growth in test tubes (9% NaCl w/v). Bacteria were streaked into the same test tubes. (LDPE) films were cut into strips with dimensions of 1.5 x 1.5 cm weighed and cleaned (30 min in 70 % ethanol), air-dried for 15 min and added to petri-dishes supplemented 15 ml of mineral salt medium (MSM), and each test isolate (1 ml of suspension) was added over the (LDPE) films. Control (+ve) was (LDPE) films and (MSM) while the control (-ve) was 1 ml suspension of test isolates and (MSM). Then cultures were incubated at 30ÚC for 21 days.

Identification of the most microbial colonization of LDPE films

Molecular identification of *F.equiseti*

The fungal strain was grown using Czapek's yeast extract agar (CYA) for *Penicillim* species and V8 Juice for *Alternaria* species followed by incubation for 7 days at 28ÚC [13]. The growing culture was prepared for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by the Intron Biotechnology Company, Korea.

The fungal DNA was then sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and rRNA gene sequencing. PCR was performed using ITS1 (forward) and ITS4 (reverse) primers which were incorporated in the reaction mixture.

Primers have the following composition: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). The purified PCR product (amplicons) was sequenced with the same primers with the incorporation of ddNTPs in the reaction mixture[14]. The obtained sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was implemented with the help of Meg Align (DNA Star) software version 5.05.

Molecular identification of *B. parabrevis*

Bacterial strain was cultured in sterile test tubes containing 10 ml of nutrient broth medium[9]. Culture was incubated at 28ºC for 48 hours.

Patho-gene-spin DNA/RNA extraction kit provided by the Intron Biotechnology Company, Korea was used. The extracted DNA samples were sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and gene sequencing. PCR was performed using two universal primers namely 27F (5'-AGAGTTTGATCC TGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3').

The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoresis on 1% agarose gel. Purified amplicons were sequenced in the sense and antisense directions using 27F and 1492R primers with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture[14]. Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was carried out using Meg Align (DNA Star) software version 5.05.

Measurement of biodegradation

I. Visual observation (microbial attack the plastic surface bio-film or hyphae penetration formation).
II. SEM in order to check for any changes in surface morphology.
III. FTIR analysis to detect the degradation on the basis of changes in the functional groups.

(SEM) analysis

The positive control and the treatment were prepared for (SEM) analysis (washing with70% ethanol). The samples were pasted onto the (SEM) sample stub using a carbon tape and the sample was coated with gold and analyzed under high-resolution scanning electron microscope (Quanta FEG 250, FEl, USA) at Desert Research Center, Cairo, Egypt.

(FTIR) analysis

Fourier transform infrared spectroscopic analysis was performed for the control and the treatment. The analysis was performed using Perkin-Elmer Spectrum version 10.5.4 at Central lab, Faculty of Science, Helwan University, Cairo, Egypt.

IV. Weight loss measurement (determine of residue polymer)

The weight loss in LDPE film percentage calculated using the equation 1:

Weight loss in (LDPE) (%) = (Initial Weight – Final Weight) / Initial Weight) x 100[15].

Where: Initial weight= before treatment.

Final weight= after treatment with *F.equiseti* and *B.parabrevis*.

Optimisation of the biodegradation potential of LDPE films by *F.equiseti* and *B.parabrevis*

The growth conditions effect such as media types, inoculum size, shaking speed, incubation temperature, pH at different incubation time were studied.

Effect of media types

Plastic film

There are two types of broth media used, namely, Czapek-Dox broth and mineral salt media (MSM). One disk (8 mm in diameter) of *F.equiseti* was inoculated into each of three sets of autoclaved flasks (250 ml) containing 50 ml of broth supplemented with sterilized LDPE films (1.5 cm x 1.5 cm), then it was incubated at 30ÚC for 21 days. After incubation, the dry mycelium weight was determined through a fungal mycelium filtration by using vacuum filtration, then dried mycelia were weighed by using digital balance, and weight loss in the (LDPE) films percentage was also calculated. On the other hand, in the case of *B.parabrevis*, one ml (bacterial suspension) was inoculated into each of the three sets of autoclaved mineral salt media, separately supplemented with sterilized (LDPE) films (1.5 cm x 1.5 cm) as shown in Pictures (4A & 4B), then incubated at 35ÚC for 21 days. After incubation, the bacterial growth was determined at 600 nm spectrophotometrically and weight loss in the LDPE film (%) was calculated. Positive control was media + plastic film (LDPE), while the negative control was media+ inoculum (*B.parabrevis* or *F.equiseti*).

Effect of inoculum size changes

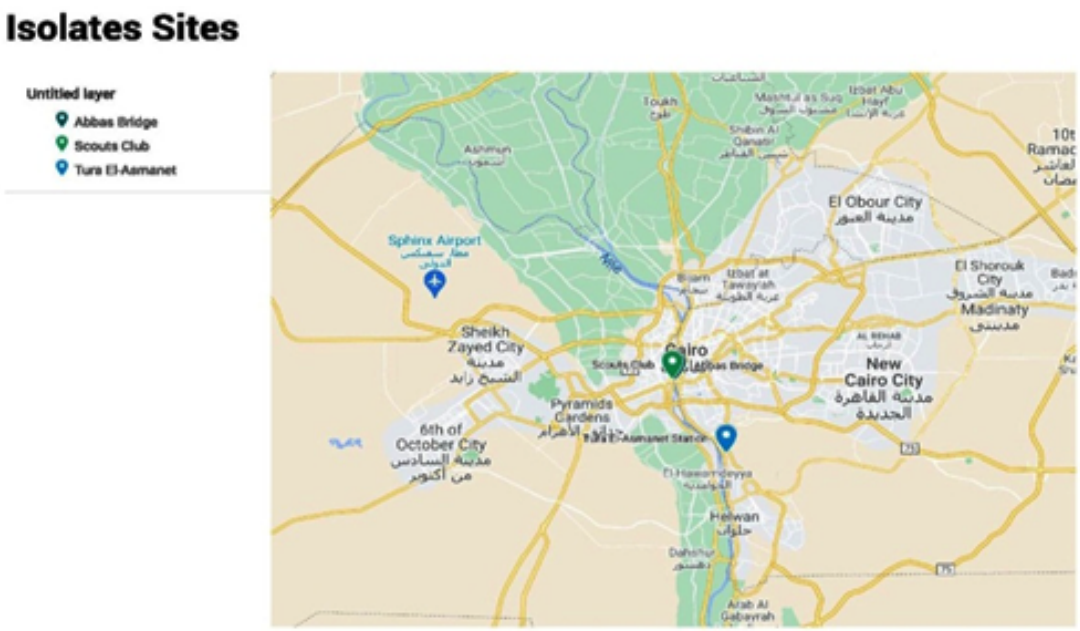
Different inoculum size of *F.equiseti* (1, 2, 3 disks with 8 mm in diameter) and *B. parabrevis* (1, 2, 3 ml bacterial suspension) in three sets of 250 ml contained 50 ml (MSM) were supplemented with sterilized (LDPE) films (1.5 cm x 1.5 cm), then incubated at 30ÚC and 35ÚC for *F.equiseti* and *B.parabrevis*, respectively, for 21 days. After incubation, weight loss in (LDPE) films percentage was calculated.

Effect of shaking speed and static

Mineral salt media were supplemented with sterilized films and dispensed in three sets of 250 ml flasks containing 50 ml, and then the flasks were inoculated with two ml *B.parabrevis*. *F. equiseti* inoculated with two disks (8 mm in diameter) and incubated at both static and shaking speed (150 rpm) for 21 days at 30ÚC and 35ÚC for *F. equiseti* and *B.parabrevis*, respectively. After the end of the incubation, weight loss in the (LDPE) films percentage was calculated.

Effect of incubation temperature at different incubation times

Fifty ml of liquid (MSM) with one piece



Picture 1. Isolates Sites (www.google.com/maps)

of LDPE film (1.5 cm x 1.5 cm) was prepared and filled into in 250 ml flasks, and it was incubated at different temperatures 25, 30, 35, and 40 ÚC in intervals of 5 days (i.e. 5, 10, 15, and 20 days) for *B.parabrevis* while in the case of *F.equiseti*, the intervals include 7, 14, 21, and 30 days. Optimal temperature was employed depending on the weight loss in (LDPE) films percentage.

Effect of pH at different incubation times

The ability of *B.parabrevis* and *F.equiseti* to utilized (LDPE) as a sole source of carbon and energy, (MSM) with one piece of (LDPE) at different pH values (i.e. 3, 5, 7 & 9) was adjusted using phosphate and acetate buffers. Then the cultures were incubated at 30ÚC and 35ÚC for 21 days and 30 days for *F.equiseti* and *B.parabrevis*, respectively. The weight loss in (LDPE) films percentage was calculated after the incubation.

RESULTS AND DISCUSSIONS

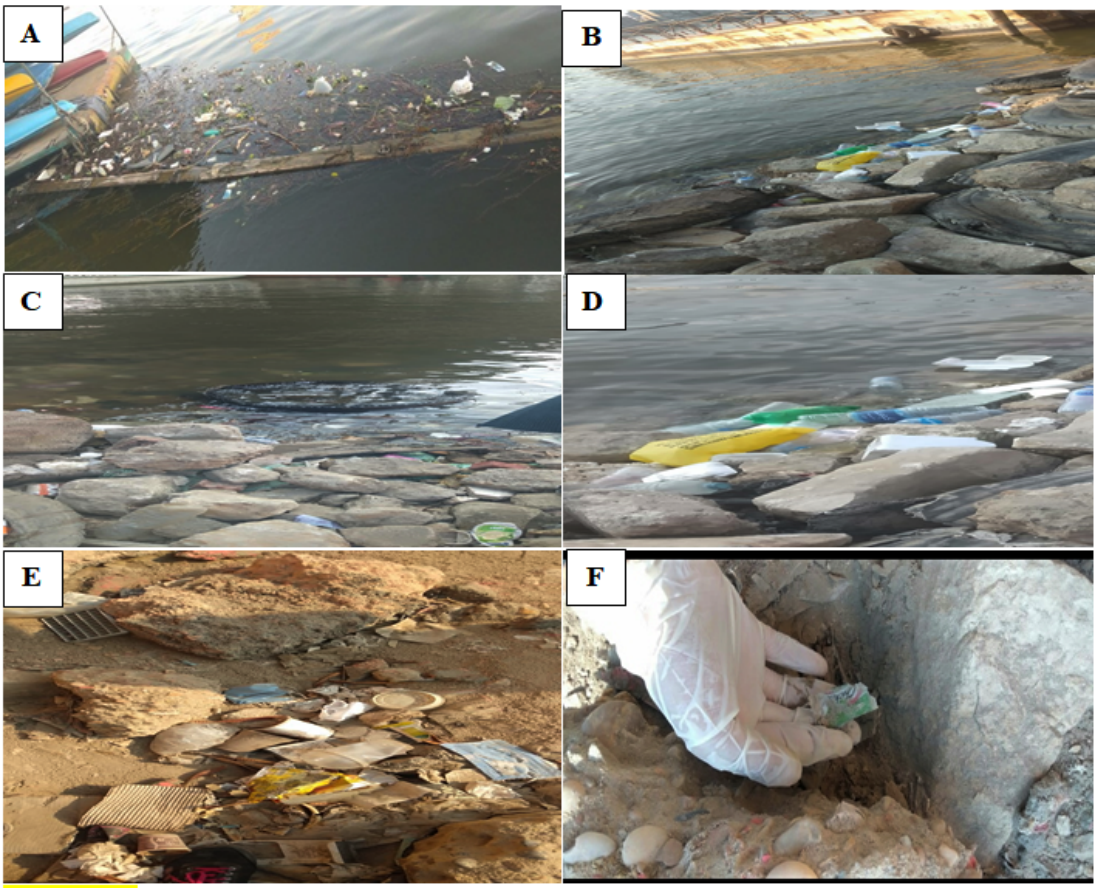
Isolation of micro-biota

A total of fifteen, micro-biota were isolated from the soil dumping of plastic debris and water polluted of plastic. The isolates were labelled as 1S through 15 S. PDA was used for fungal isolation and nutrient agar for bacterial isolation as shown in Table (1). The first seven, micro-biota was isolated from the water polluted of plastic, and the other eight, micro-biota were isolated from the soil dumping of the plastic debris.

Screening of the isolated micro-biota biodegradation potential for low density polyethylene films

Plastic film

Screening micro-biota biodegradation potential was determined by visible observation



Picture 2. Collection sites (2A-2D) Abbas Bridge and Scout Club, (2E-2F) Tura El-Asmanet Metro Station, Cairo, Egypt

through the growth observed above LDPE film as attack with the fungi as shown in Pictures (5A-5C) and bacteria isolates. The results in Table (2) show that the fungal isolate named 9S and the bacterial isolate named 1S achieved a positive visual observation result when the growth of these two isolates was observed compared to other isolates after 21 days of incubation. These results agreement with Merina & Santosh[16] that indicate, the formation and attachment of a biofilm on LDPE film. On the other hands, the microbial colonization on polymer surface is the first requirement for its biodegradation [17, 18].

Picture 3. Water samples were collected from the surface through sterile glass bottle (Screw Cap bottles) and stored until used at 4°C

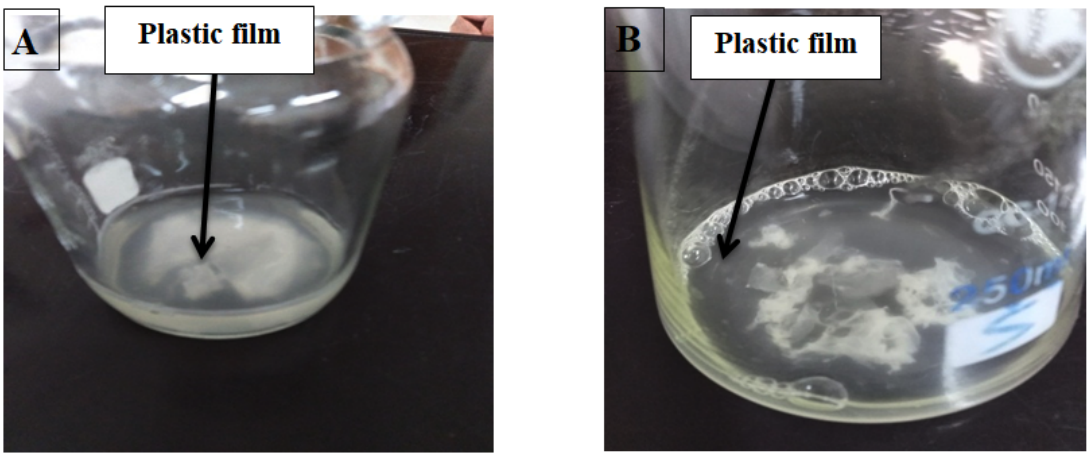
Identification of the most microbial colonization of LDPE films shown in figures (1, 2)

Sample *Fusarium sp1*: *Fusarium equiseti* AUMC15175 (534 letters)

C C T G C G G A G G G A T C A T T A C C G A
G T T T A C A A C T C C C A A A C C C C T G T G A
A C A T A C C T A T A C G T T G C C T C G G C G G A
T C A G C C C G C G C C C C G T A A A A A G G G A C
G G C C C G C C C G A G G A C C C C T A A A C T C T G T T T
T T A G T G G A A C T T C T G A G T A A A A C A A A C A A
A T A A A T C A A A A C T T T C A A C A A C G
G A T C T C T T G G T T C T G G C A T C G A T

Table 1. Summary of the microbial species isolated from the plasticized polluted sites

Microbial isolates	Source	Identification
1S	Water	*Bacteria sp1*
2S	Water	*Aspergillus sp1*
3S	Water	*Alternaria sp*
4S	Water	*Trichoderma sp*
5S	Water	*Cladosporium sp*
6S	Water	*Aspergillus sp2*
7S	Water	*Bacteria sp2*
8S	Soil	*Bacteria sp3*
9S	Soil	*Fusarium sp1*
10S	Soil	*Aspergillus sp3*
11S	Soil	*Penicillum sp1*
12S	Soil	*Penicillum sp2*
13S	Soil	Rhizopus *sp*
14S	Soil	*Apergillus sp4*
15S	Soil	*Fusarium sp2*



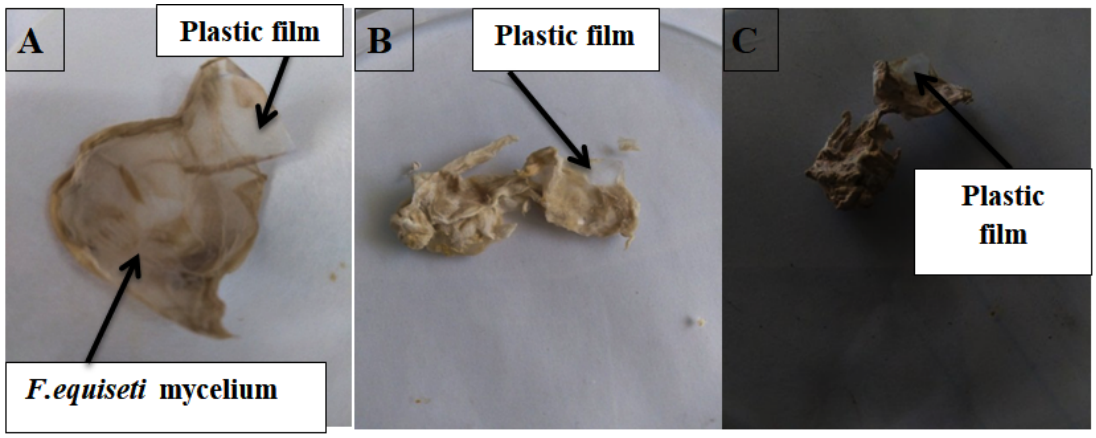
Picture 4. The bacterial inoculation MSM supplemented with sterilized LDPE films (1.5 cm x 1.5 cm) (4A, 4B)

G A A G A A C G C A G C A A A A T G C G A T
A A G T A A T G T G A A T T G C A G A A T T C
AGTGAATCATCGAAT CTTTGAACGCACAT
TGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAAC
C C T C A A G C T C A G C T T G G T G T T G G
G A C T C G C G G T A A C C C G C G T T C C
C C A A A T C G A T T G G C G G T C A
C G T C G A G C T T C C A T A G C G T A G
T A A T C A T A C A C C T C G T T A C T G
G T A A T C G T C G C G G C C A C G C C G T A
A A A C C C C A A C T T C T G A A T G T T G
A C C T C G G A T C A G G T A G G A A T A C C C G

CTGAACTTAAGCATATC AATAAGCGGAGGA

Sample Bacteria sp1 *Brevibacillus parabrevis* AUMC-B1 (1405 letters)

A A G T C G A G C G A G G G T Y T T C G
G A C C C T A G C G G C G G A C G G G T G A G T
A A C A C G T A G G C A A C C T G C C T C T C
A G A C C G G G G A T A A C A T A G G G A A A C
T T A T G C T A A T A C C G G A T A G G T T T
T T G G A T T G C A T G A T C C G A A A A G A
A A A G A T G G C T T C G G C T A T C A C T G
G G A G A T G G G C C T G C G G C G C A T T A
G C T A G T T G G T G G G G T A A C G G C C T A C C A A G
G C G A C G A T G C G T A G C C G A C C T G A



Picture 5. LDPE film attack with *F.equiseti* mycelium (5A, 5B, 5C)

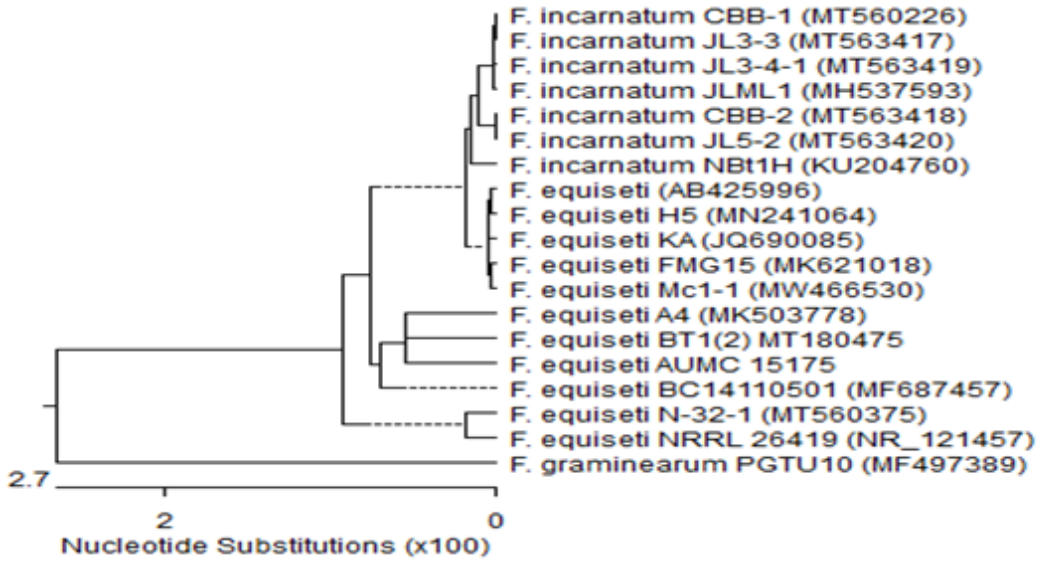


Fig. 1. Phylogenetic analysis of *F.equiseti*. Aligned closely related strains of *Fusarium* accessed from the GenBank. Sample AUMC15175 showed 99.65% to 100% identity and 99% - 100% coverage with several strains of *Fusarium equiseti*

GAGGGTGACCGGCCACACTGGG
ACTGAGACACGGCCCA GACTCCTAC
GGGAGGCAGCAGTAGGGAATTT
TCCACAATGGACG AAAGTCTGATGGAGCA
ACGCCGCGTGAACG ATGAAGGTCTTCGGA
TTGTAAAGTTCTGTTGTCAGGGAC
GAACACGTGCCGTTCGAATAG
GGCGGTACCTTGACGGTACCTG
ACGAGAAAGCCACGGCTAACTACGT
GCCAGCAGCCGCGGTAATA CGTAGGT
GGCAAGCGTTGTCCGGATTTATT
GGGCGTAAAGCGCG CGCAGGCGGCTATGT
AAGTCTGGTGTTAAAGCCCGGAG
CTCAACTCCGGTTCGCATCGGAAACTG
TGTAGCTTGAGTGCAGAAGAGGAA
AGCGGTATTCCACGTGTAGCGG
TGAAATGCGTAGAGATGTGGA
GGAACACCAGTGGCGAAGGCGGCTTT
CTGGTCTGTAACTGACGCTGA
GGCGCGAAAGCGTGGGGAG
CAAACAGGATTAGATACCCTGGTAG
TCCACGCCGTAAACGATGAGTGC
TAGGTGTTGGGGGTTTCAATA
CCCTCAGTGCCGCAGCTAACGCAA
TAAGCACTCCGCCTGGGGAGTA
CGCTCGCAAGAGTGAAACT
CAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGA
AGAACCTTACCAGGTCTTGA
CATCCCGCTGACCGCTCTGGAGAC
AGAGCTTCCCTTCGGGGCAGC
GGTGACAGGTGGTG CATGGTTGTCGTCA
GCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGC
AACCCTTATCTTTAGTTGCCAGCAT
TCAGTTGGGCACTCTAGAGAG
ACTGCCGTCGACAAGACGGAGGA

Table 2. Screening of the isolated micro-biota biodegradation potential for low density polyethylene films.

Isolated Micro-biota	Visual observation
Bacteria sp1	+ve
Aspergillus sp1	-ve
Alternaria sp	-ve
Trichoderma sp	-ve
Cladosporium sp	-ve
Aspergillus sp2	-ve
Bacteria sp2	-ve
Bacteria sp3	-ve
Fusarium sp1	+ve
Aspergillus sp3	-ve
Penicillum sp1	-ve
Penicillum sp2	-ve
Rhizopus *sp*	-ve
Apergillus sp4	-ve
Fusarium sp2	-ve

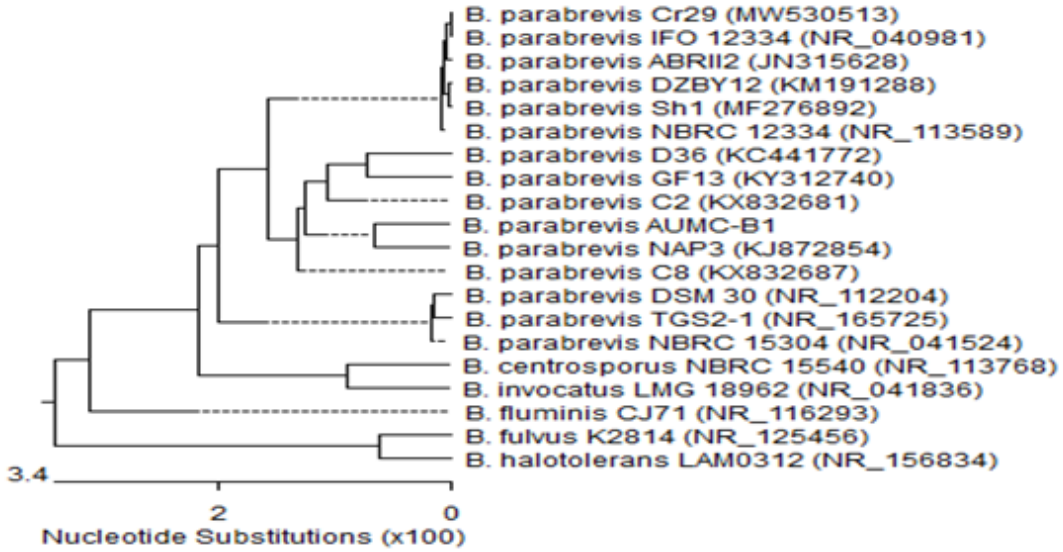
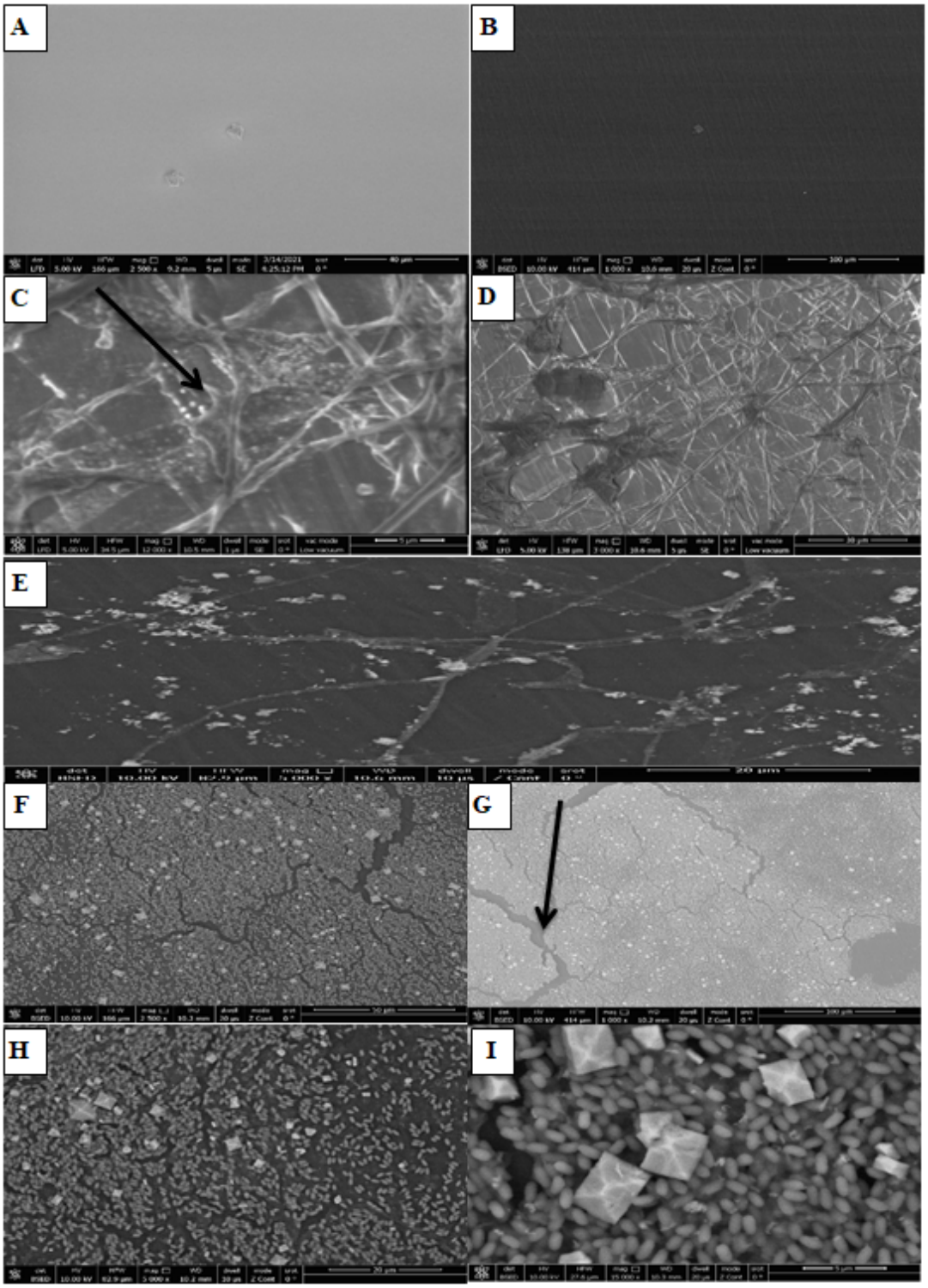


Fig. 2. Phylogenetic analysis of *B. parabrevis*. Aligned closely related sequences of *Brevibacillus* accessed from the GenBank. The bacterial strain sequenced in the current study showed 99.79% - 99.93 % identities with *Brevibacillus parabrevis* with percentage coverage of 100%



Picture 6. SEM analysis of (LDPE) film, control (6A, 6B), (LDPE) treatment with *F.equiseti* (6C, 6D, 6E), and LDPE treatment with *B.parabrevis* (6F, 6G, 6H, 6I)

A G G C G G G G A T G A C G T C A A A T C CCAATCTCTGAAAACCAATCTCAGTT
ATCATGCCCCTTATGACCTGGGC CGGATTGTAGGCTGCAACTCG
TACACACGTGCTACAATGGTTGGTACA CCTACATGAAGTCGGAATCGCTAGTA
ACGGGATGCTACCTCGCGAGAGGACG ATCGCGGATCAGCATGCCGCGGTGAA

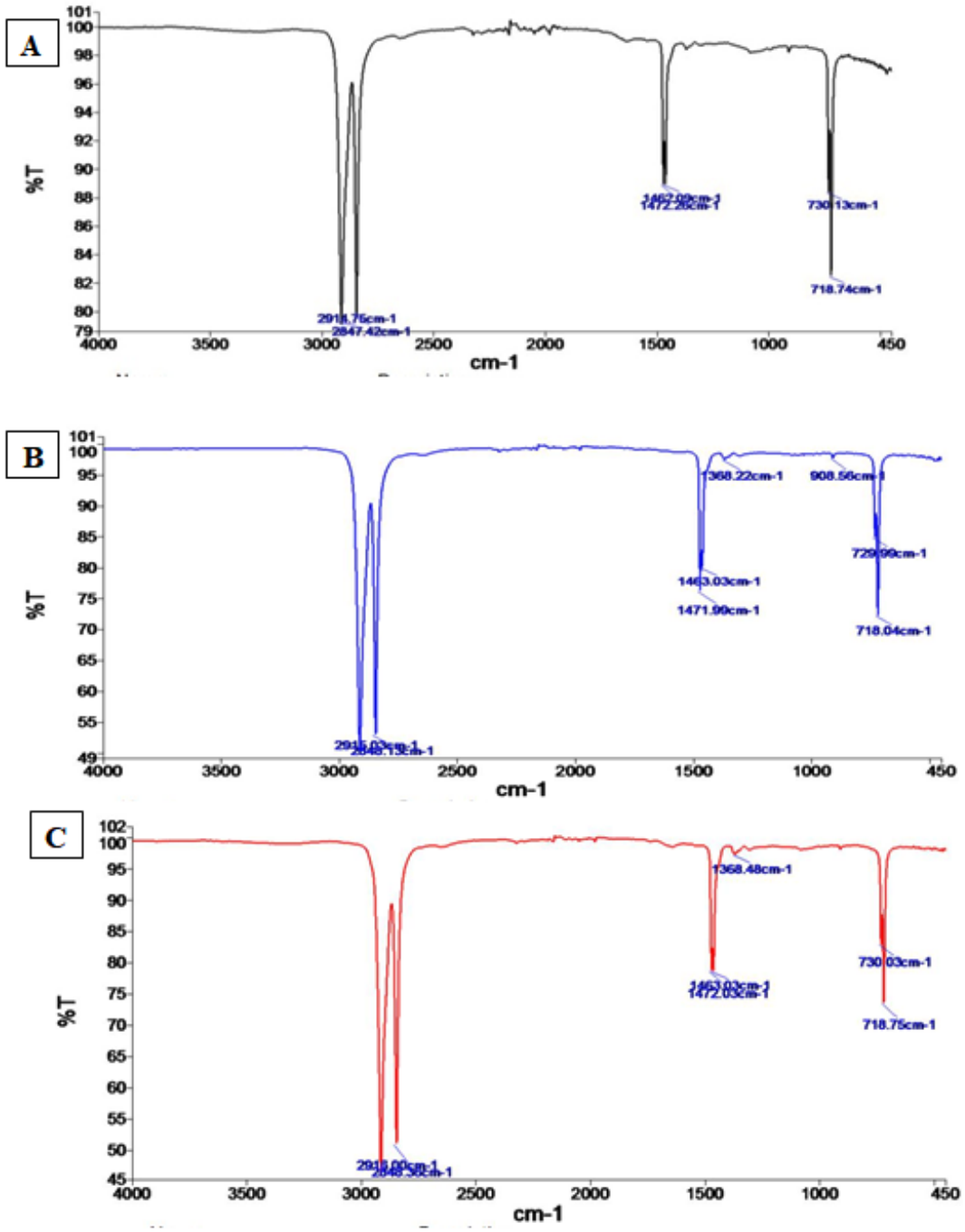


Fig. 3. FTIR analysis, (3A) control (LDPE film only), (3B-3C) (LDPE) film treatment with *F.equiseti* and *B.parabrevis,* respectively

TACGTTCCCGGGCCTTGTACACACCGC
C C G T C A C A C C A C G G G A G T T T G C
A A C A C C C G A A G T C G G T G A G G T A A C C
GCAAGGAGCCAGCCGCCGAAG

Measurement of biodegradation

SEM analysis

Picture (6) shows SEM of LDPE film surface before and after 21 days of incubation with *F.equiseti* and *B.parabrevis*. Before, the sample had a smooth surface with no pits, cracks or any attached on the surface (Pic. 6A, 6B). However, after incubation with *F.equiseti* and *B.parabrevis*, surface with defects and changes was observed (Pic. 6C, 6D, 6E, 6F, 6G, 6H & 6I). For both *F.equiseti* and *B.parabrevis*, the different places on the surface of LDPE film colonized forming

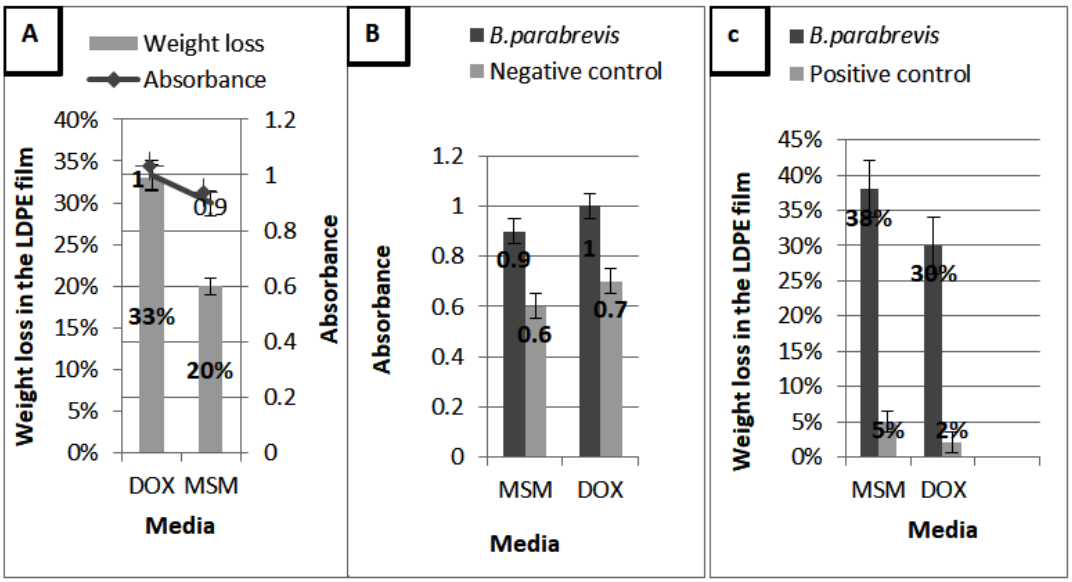


Fig. 4. Effect of media types on Weight loss (¬%) in the (LDPE) film percentage and Absorbance (¯f&¯) (4A), Absorbance (4B), Weight loss in the (LDPE) film (4C) by *B.parabrevis* (%). Error bars represent the SD of triplicates.

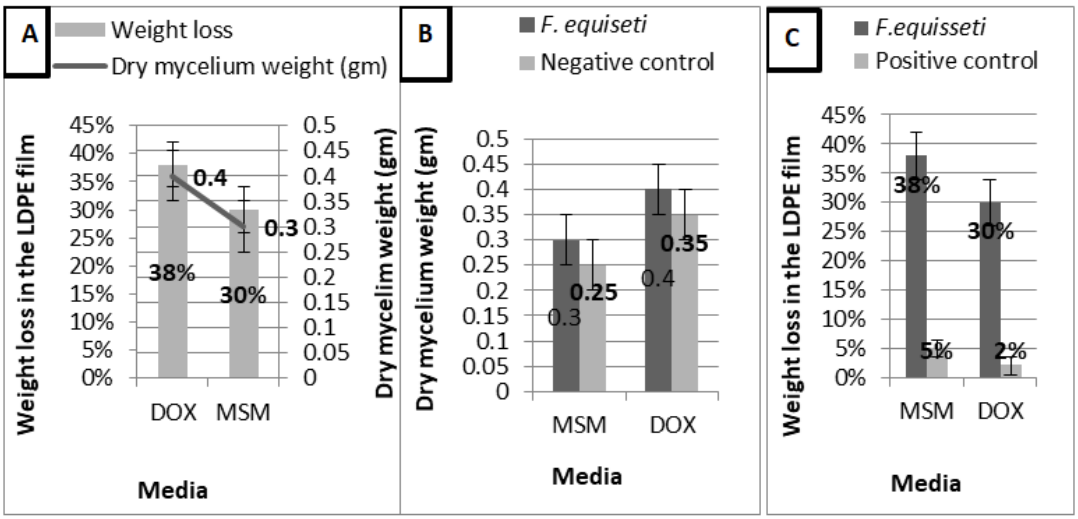


Fig. 5. Effect of media types on Weight loss (¬%) in the (LDPE) film percentage and Dry mycelium weight (¯) (5A), Dry mycelium weight (5B). Weight loss in the LDPE film (8C) by *f.equiseti*(%). Error bars represent the SD triplicates

biofilm; this proved its strong adhering capabilities as well as LDPE utilization capacities.

These results are similar to those obtained by Merina and Santosh [16]. In the case of *F.equiseti*, it was found the hyphal growth over LDPE film surface and hyphal penetration (Pic. 6C, 6D & 6E) while in the case of *B.parabrevis*, several cracks and cavities on the surface developed in addition to the biofilm formation were observed (Pic. 6F, 6G, 6H & 6I). Our results are agreed with[11].

FTIR analysis

There was a variation in the intensity of bands after incubation with *F.equiseti* and *B.parabrevis*) compared with control as shown (Fig. 3A, 3B & 3C). For control spectrum, bands were assigned at 2915.03, 2848.13 cm^{-1} (both due to C-H stretch), 1471.99, 1463.03, 1368.22 cm-1 (C=C stretch), 908.56 cm^{-1} (C-H alkenes out of plane bend), and 729.99, 718.04 cm^{-1} (C-H bend-mono). Similar changes were observed in *F.equiseti* and *B.parabrevis*. The band at 1368.22 cm^{-1} corresponds to C=C and the band at 908.56 cm^{-1} corresponds to C-H alkenes that has been disappearing in the case of *F.equiseti* and *B.parabrevis* , this confirms the depolymerization activity of *F.equiseti*. These results are similar to

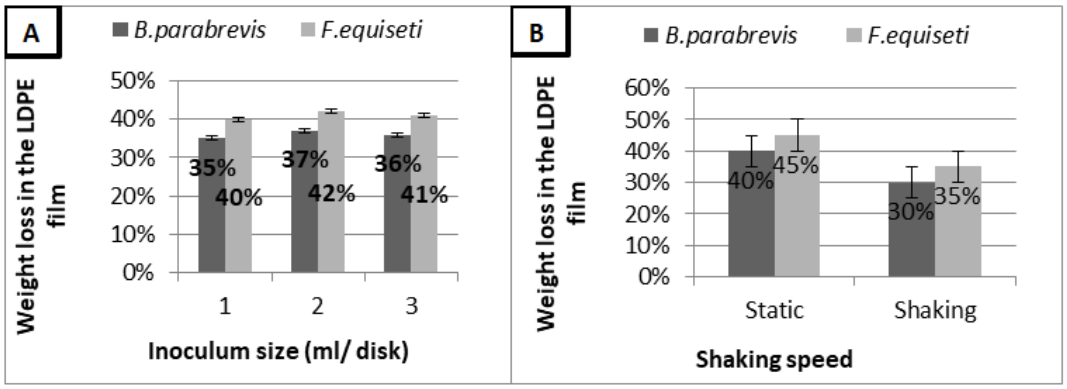


Fig. 6. Effect of inoculum size (6A), Effect of shaking and static (6B) with *B.parabrevis* (%) and *f.equiseti* (¡%). Error bars represent the SD triplicates

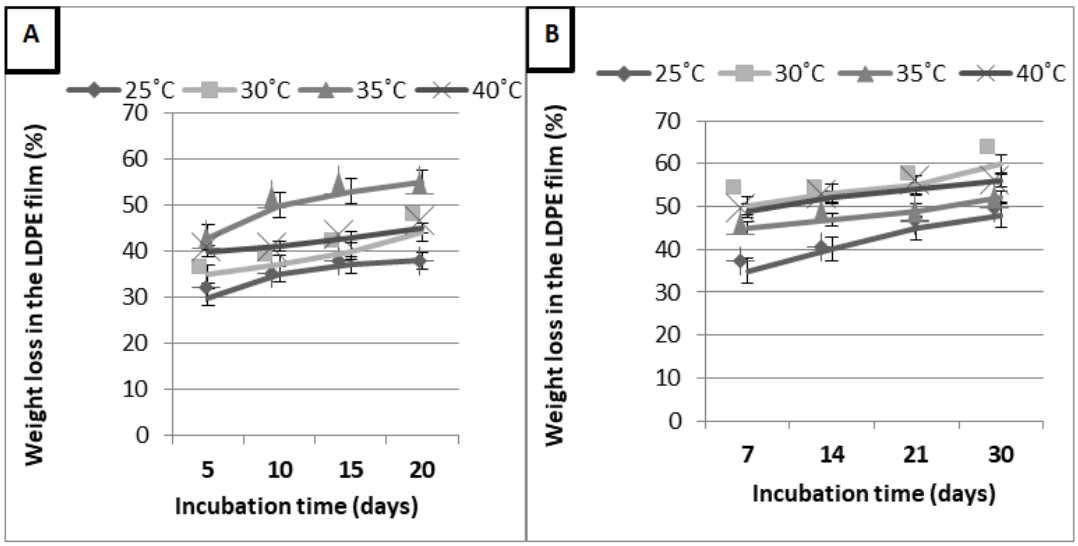


Fig. 7. Effect incubation temperature (25°C ¯f&¯, 30°C¯ %¯, 35°C ¯²%¯, 40°C ¯›‹¯) on weight loss in the LDPE film by *B.parabrevis* (7A) and by *F.equiseti* (7B) at different incubation time. Error bars represent the SD of triplicates.

those obtained by Merina and Santosh[16]. Moreover, the band appeared at 729.99 cm^{-1} for the control, and a shift in the band was observed at 730.13, 730.03 cm^{-1} in the sample treated by *F.equiseti* and *B.parabrevis*, respectively. Nupur et al[18] carried out a similar study and observed that, the band appeared at control film and a shift in (LDPE) film, which was subjected to microbial treatment degradation of polyethylene by the fungal consortium.

Optimisation of the biodegradation for LDPE films by *F.equiseti* and *B.parabrevis*

Effect of media types

The obtained results in Figures (4A- 4C & 5A-5C) show that the weight loss in the (LDPE) films % was achieved using (MSM) whereas the optimum absorbance for *B.parabrevis* and the optimum dry mycelium weight for *F.equiseti* were achieved using Dox's media, compared to positive and negative control.

Therefore, (MSM) is chosen to optimize the weight loss in the (LDPE) films percentage. This observation may go back to MSM's defect to carbon source while Dox's media contain carbon source which retards (LDPE) film as a source of carbon.

Effect of inoculum size changes

The results in Figure (6A) reveal that the best inoculum size at which the highest weight loss in (LDPE) films percentage for *B.parabrevis* and *F.equiseti* was achieved was two ml and two disks, respectively.

In addition, there was not a significant difference between two and three ml/disks. Thus, two ml/disks were chosen for optimizing the weight loss in (LDPE) films.

Effect of shaking speed and static

The results in Figure (6B) show that the optimum weight loss in LDPE was achieved at static condition compared to shaking conditions after incubation for 21 days using MSM media with the following values: 40% and 45% for *B.parabrevis* and *F.equiseti*, respectively. These may be due to static conditions allowing the (LDPE) colonization by *B.parabrevis* and *F.equiseti* incontast shaking conditions.

Effect of incubation temperature at different incubation times

The results presented in Figures (7A, 7B) show that the optimum weight loss in (LDPE) film percentage was observed at 35ÚC for *B.parabrevis* after incubating for 20 days while in the case of *F.equiseti*, it was at 30ÚC for 30 days.

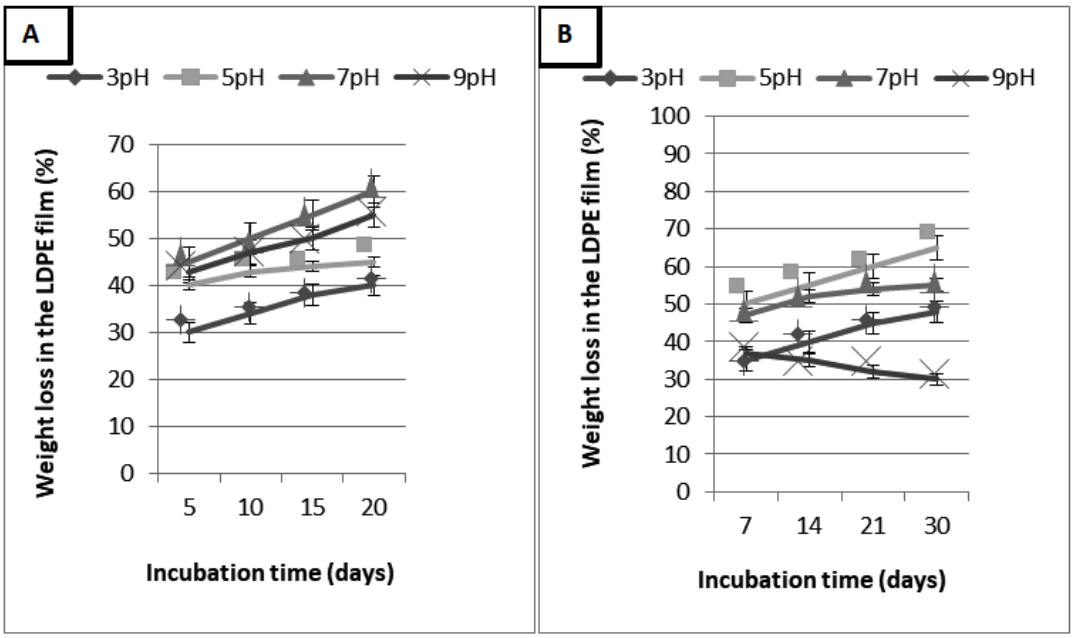


Fig. 8. Effect of pH (pH3¯f&¯, pH5 ¯ %¯, pH7¯²%¯, pH9 ¯›‹—) on weight loss in the LDPE film *B.parabrevis* (8A) and by *F.equiseti* (8B) at different incubation time. Error bars represent the SD of triplicates

Effect of pH at different incubation times

The results in Figures (8A, 8B) demonstrate that there is no significant difference between pH5 and pH7 for *F.equiseti*. Hence, pH5 was chosen to optimize the weight loss in LDPE film percentage by *F.equiseti* while in the case of *B.parabrevis*, there is no significant difference between pH 7 and pH 9. Therefore, pH7 was chosen to optimize the weight loss in (LDPE) film percentage by *B.parabrevis*. Longer incubation times revealed no significant difference between the weight losses in (LDPE) film percentage.

CONCLUSIONS

The present study gives the evidence for biodegradation of (LDPE) films. Here fifteen, micro-biota were isolated from soil dumping of plastic debris and water polluted of plastic and identified at a genus level.

Then there was a screening for (LDPE) film biodegradation using (MSM). Two isolates achieved the highest surface colonization of (LDPE) films, identifying molecular to *F.equiseti* and *B.parabrevis*. Measurement of biodegradation using (SEM) and (FTIR) analysis show the attachment or adhering of *F.equiseti* and *B.parabrevis* to the surface of (LDPE) and difference in functional groups. Furthermore, optimization the weight loss in (LDPE) film percentage was performed through different growth parameters such as media, inoculum size, shaking, incubation temperature, pH at different incubation times, and the results showed that the highest weight loss in (LDPE) films percentage reached to 60% and 65% by *B.parabrevis* and *F.equiseti*, respectively.

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Future prospects

Further study is required to get more supportive data for degradation mechanism.

Ethical approval

Not applicable.

Author's contributions

Dr. Sally A. Ali is a major contributor in writing the manuscript. She performed the practical part of the manuscript which includes the experimental methods and data reported on results, and participated in writing the manuscript; also, she is the corresponding author. All authors read and approved the final manuscript. Miss Shimaa Zakarya collected the samples. Miss Shimaa Khaled revised the manuscript.

Conflict of interest

The authors declare that they have no conflict of interests.

Availability of data

The data generated or analyzed during this study is included in this published article (and its supplementary information files).

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