Biodegradation of Low Density Polyethylene (LDPE) by Mangrove Fungi From the Red Sea Coast

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SUMMARY

Forty five fungal isolates belonging to 13 genera were derived from tidal water, floating debris, and sediment collected from mangrove stands on the Red Sea coast of Saudi Arabia. Six of these isolates and their consortium were found to be able to grow in association with low density polyethylene (LDPE) film under in vitro conditions in the absence of dextrose or any other carbon source. These isolates were further tested for their potential to degrade LDPE by co-cultivation under aeration on a rotary shaker. Examination under light and scanning electron microscope revealed that the fungi attached themselves to the surface of the film and grew profusely. As compared to the controls, these fungi accumulated significantly higher biomass, produced more ligninolytic enzymes, and released larger volumes of CO_2 during co-cultivation with LDPE. These observations indicated that the selected isolates were able to breakdown and consume the LDPE film.

Keywords: Biodegradation, Mangroves, LDPE, *Alternaria alternata*, *rDNA*

INTRODUCTION

About 140 million tons of synthetic polymers are produced worldwide annually with their utility escalating at a rate of 12% per annum [1].

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Saudi Arabia is one of the major producers of plastics in the world, with total production of around six million tons per year [2]. The share of plastic wastes is around 15% of the total municipal waste in Saudi Arabia [2].

There are reports that suggest that polyethylene causes blockages in the intestines of fish, birds and marine mammals. In addition, entanglement in or ingestion of this waste has endangered hundreds of different species [3, 4]. Since plastic wastes pose a serious threat to health and environment, increasing efforts are being devoted to discover fungi and other microorganisms, which can decompose these polymers and neutralize their potential harmful effects.

Recently, efforts have focused on the biodegradation of polyethylene (LDPE) waste due to the disadvantages of other methods, such as cost and pollution. Biodegradation is the ability of microorganism to influence abiotic degradation through physical, chemical or enzymatic action [5].

It is evident from the recent studies that the speed of biotic degradation of low-density polyethylene (LDPE) can be enhanced by its prior oxidation [6-9]. It is probable that the oxidation of polyethylene generates carbonyl groups that can be used by microorganisms for its degradation [10-12].

Kumar et al. [13], isolated bacteria from tropical mangrove soil, which had the ability to decompose low-density polyethylene polymers. Yirui [14], isolated fungi from mangrove sediment soil to decompose polycyclic aromatic hydrocarbons (PAHs). Gofar [15], isolated species of *Aspergillus* and other fungi, which showed potential to degrade petroleum hydrocarbons. Esmaeili et al. [16], used mixed culture of *Aspergillus niger* and *Lysinibacillus xylanilyticus* to test its efficacy for biodegradation of LDPE. Fungi produce enzymes, which can metabolize complex organic compounds; and that is why, their role in biodegradation has been studied extensively [17, 18]. Several studies have investigated the polyethylene biodegradation process using fungal isolates, such as *Aspergillus terreus, A. fumigatus* [19[and *A. flavus* [20].

The present study aims to (a) isolate fungal strains from the major mangrove stands on the Red Sea coast of Saudi Arabia; (b) to detect the Potential of these isolates to degrade low-density polyethylene (LDPE); (c) to compare fast degradation among strains and their consortium and (d) the extracellular enzyme system involved.

MATERIALS AND METHODS

Collection of Samples

Samples of tidal water, floating debris, and sediment surrounding the pneumatophores were collected in sterilized tubes. The debris consisted of dead fallen leaves, pieces of pneumatophores, bark and wood. The samples were taken from three mangrove stands south Corniche of Jeddah; South Coast of Jazan on the South Coast of Saudi Arabia and Farasan Island 40 km off the shore. The samples were brought to lab in ice box for isolation of fungi.

Isolation of Fungi

Isolation of fungi from tidal water was done by serial dilution. Ten-fold dilutions were laid a synthetic medium formulated to simulate seawater conditions based on the compositions of Ameen et al. [21]. Composition of the medium per liter was: $MgSO_4$ 246.5 mg, $FeSO_4.7H_2O$ 5.56 mg, $ZnSO_4.7H_2O$ 0.29 mg, $MnSO_4.H_2O$ 0.34 mg, $CuSO_4.5H_2O$ 0.025 mg, NH_4Cl 5.35 mg, KCl 7.46 mg, $CaCl_2.2H_2O$ 1.47 mg, NaCl 5.84 mg, $COCl_2.6H_2O$ 0.027 mg, KH_2PO_4 136 mg, Na_2MoO_4 24 mg with pH adjusted to 5.5. Standard quantity of dextrose (20 g/l) was added as source of carbon; and the medium was gelled with 15 g agar per liter. For the sake of convenience, the medium was tentatively designated as 'MF1'.

Fungi from solid debris were isolated by surface sterilizing the specimens followed by splitting into smaller pieces and directly laying on 'MF1' medium.

Dilution technique was used for isolation from sediments also. One gram of sediment was suspended in 10 ml of sterilized distilled water; and ten-fold dilutions thereof were spread on the same medium. The plates were incubated at 30 ± 2ºC for 5-7 days.

Identification of the Fungi

Pure fungal isolates were established from the initial plates using standard procedures of fungal cultivation. The isolates were examined under the microscope and were identified according to the procedures of Barnett and Hunter [22].

For precise identification of the isolates, 18S rDNA gene fragment was amplified by PCR using ITS1 and ITS4 primers as described by White et al. [23]. The PCR products were cleaned using Ultra Clean® PCR Cleanup Kit

(MoBio, USA), and electrophoresed on Genetic Analyzer AB-3130 (Applied Biosystems) and sequence data was generated using Gene Mapper Version 4.0 (AB). Sequences were compared and analyzed against the database of National Center for Biotechnology Information (NCBI) using the BLAST program. A dendrogram of phylogenetic relationships was constructed by neighbor-joining algorithm using maximum composite likelihood model.

Fungal Growth and Biomass Accumulation on LDPE

In all, 45 isolates derived from different types of samples were cultivated with LDPE film pieces to test activity on the film. 'MF1' broth devoid of dextrose or any other source of carbon was used for the tests. LDPE pieces of 1 cm^2 were cut from sterilized polyethene film of density 0.92 gcm^{-3}. Single pieces of LDPE were precisely weighed and transferred to 150 ml conical flasks containing 100 ml sterilized 'MS1' broth. Fungal discs of 6 mm diameter were punched out from the culture plates with the help of sterilized steel tube. Each flask was inoculated with a single disc of an individual isolate. The flasks were incubated at 30 ± 2°C for 4 weeks in a rotary shaker gyrating at 100 rpm. Three replicate flasks were maintained for each fungal isolate. For each treatment, three flasks with corresponding inoculum but without LDPE pieces were retained as controls. All the isolates were tested for activity against LDPE; but on the basis of superior growth, six isolates namely, *Aspergillus caespitosus, Phialophora alba, Paecilomyces variotii, Aspergillus terreus, Alternaria alternata* and *Eupenicillium hirayamae*, and their consortium were included in subsequent tests.

Biomass accumulation of the selected isolates was estimated in comparison with the controls after four-week incubation. LDPE film pieces with fungal growth were dried in the oven at 90ºC overnight and were weighed precisely again. Gain in biomass under each treatment and the corresponding control was recorded by subtracting the initial weights; and difference between gain in treatment and control was considered to be due biodegradation activity of the fungus. Consortium of the isolates under test was also included in the experiment.

Light Microscopy of LDPE Film

Before starting the treatment with fungal isolates, surface profile of LDPE pieces was examined under light microscope. Subsequently, after incubation for four weeks, pieces of LDPE were taken out of the medium and were washed under a mild stream of water to remove overgrowth of fungus. The attached

fungus was stained with cotton blue and the pieces of film were examined to check attachment and colonization of fungal structures on the surface.

Scanning Electron Microscopy of LDPE Film

LDPE film pieces showing fungal colonization after four weeks were examined by scan electron microscopy (SEM) also along with control pieces retained before the treatment. Specimens for SEM were prepared according to Kuo [24]. Samples were immersed in 2.5% glutaraldehyde-phosphate buffer solution for 3 hours. After draining the liquid, the specimens were rinsed 3 times with sodium cacodylate buffer. The samples were post fixed in osmium tetroxide for 1 hour and rinsed with distilled water. After dehydrating the specimens through sequential ethanol series of 25%, 50%, 75%, followed by 100%, each for 10 min, these were rinsed with distilled water and were mounted on specimen stabs. Finally the specimens were coated with gold and viewed in SEM at 20 KV and T1 grain size of 40 µm and a working distance of 15 cm.

Enzyme Activity Assays

Six fungal isolates, which showed biomass accumulation under treatment and their consortium, were co-cultivated with LDPE film pieces in replicates along with the controls as above. After four weeks of incubation, enzyme level in the medium was assayed for controls and treatments. Cultures were centrifuged at 10,000 rpm, 4°C; the pellet consisting of fungal cells was discarded and enzyme level was determined in the extracellular fluids. Laccase was estimated by the oxidation of 2,2-Azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid (ABTS) according to Novotny et al. [25] and Wu et al. [26]. using 0.1 mM ABTS in the reaction buffer of 100 mM sodium tartrate (pH 4.5) with 50 µl culture filtrate. One unit (U) of laccase activity was defined as the production of 1 µmol product per min at 30°C and pH 4.5. Manganese-dependent peroxidase (MnP) was estimated by using 0.01% phenol in the presence of 0.1 mM H_2O_2 and 1 mM $MnSO_4$ in 100 mM sodium tartrate (pH 4.5); while lignin peroxidase (LiP) was determined by the oxidation of 2 mM veratryl alcohol in 100 mM sodium tartrate (pH 4.5) with 0.4 mM H_2O_2 [27]. All enzymes assayed in this study were expressed as U/ml.

Estimation of CO_2 Evolution

Six selected fungal isolates and their consortium were cultivated with LDPE film pieces as above and quantity of CO_2 evolved was estimated for an incubation

period of 4 weeks. Three flaks were maintained for each isolate together with corresponding control flasks inoculated with the fungus but devoid of film.

Volumetric and gravimetric estimation of CO_2 evolved during 4-week incubation was performed using strum test. For gravimetric analysis sterile air was sequentially passed through1M KOH solution to remove atmospheric CO_2 and then through the flasks under assessment. The bubbling air provided aeration for the fungal activity and at the same time allowed any CO_2 evolving from the fungal activity to dissolve readily in the broth. The test was performed at room temperature (26 ± 2°C). Amount of CO_2 dissolved in the broth was estimated by adding 100 ml of 0.1 M $BaCl_2$ which formed precipitate of barium carbonate; and CO_2 released was gravimetrically calculated by measuring the weight of the precipitate. Difference in the values obtained between control and test bottles were recorded.

For volumetric analysis, the dissolved carbon dioxide present in the medium was estimated by titration. The broth was filtered to remove fungal mass and the LDPE substrate and 25 ml filtrate was taken in a conical flask to which 0.05 ml of 0.1N thiosulphate solution was added. After the addition of 2 drops of methyl orange indicator, solution was titrated against 0.02M sodium hydroxide solution. End point appeared as change in color from orange red to yellow. After this two drops of phenolphthalein indicator was added and titration was continued until a pink color was observed. Volumes of the titrant used were noted and the amount of CO_2 evolved was calculated using the formula: [A x B x 50 x 1000] ÷ V; Where A = volume of NaOH titrant in ml, B = normality of NaOH, and V = volume of sample in ml

RESULTS AND DISCUSSION

Identification of Fungal Isolates

Forty five isolates were derived from the samples. These were identified as eight species each of *Aspergillus* and *Penicillium*; three species of *Candida*; while remaining ten were individual species of *Acremonium*, *Alternaria*, *Emericella*, *Eurotium*, *Exophiala*, *Geosmithia*, *Paecilomyces*, *Pichia*, *Phialophora* and *Cladosporium*.

Fungi from marine environment have been frequently isolated in the quest of novel characteristics probably because they belong to a domain drastically different from the terrestrial regimes and, therefore, are likely to possess unusual properties. Sindujaa et al. [28], isolated species of *Aspergillus* from marine water, which was showed capability of degrading polyethylene. Luo et al. [28], isolated eight *Fusarium* and two *Aureobasidium* species form

sediments of mangrove stands to test their potential to degrade dimethyl phthalate ester additives of plastic materials. Pramila and Ramesh [29] have shown that *Aspergillus* species derived from coastal sea water can significantly degrade LDPE. *Fusarium* species isolated from mangrove stand in coastal water contaminated with polyaromatic hydrocarbons (PAH) have also shown capacity to breakdown PAHs, anthracene (ANT) and benz[a]anthracene (BAA), which are known toxic and carcinogenic substances escaping to the environment with industrial effluents [26].

Sequence-based Identification

The sequences of the partial 18S rDNA gene fragments cloned from the isolated strains were compared with similar data available at the GenBank by an online alignment search. The phylogenetic tree based on a comparison of the sequences is shown in **Figure 1**. The results indicated that the partial 18S rDNA sequence of both *FA-18, FA-20, FA-24* were 100% identical to that

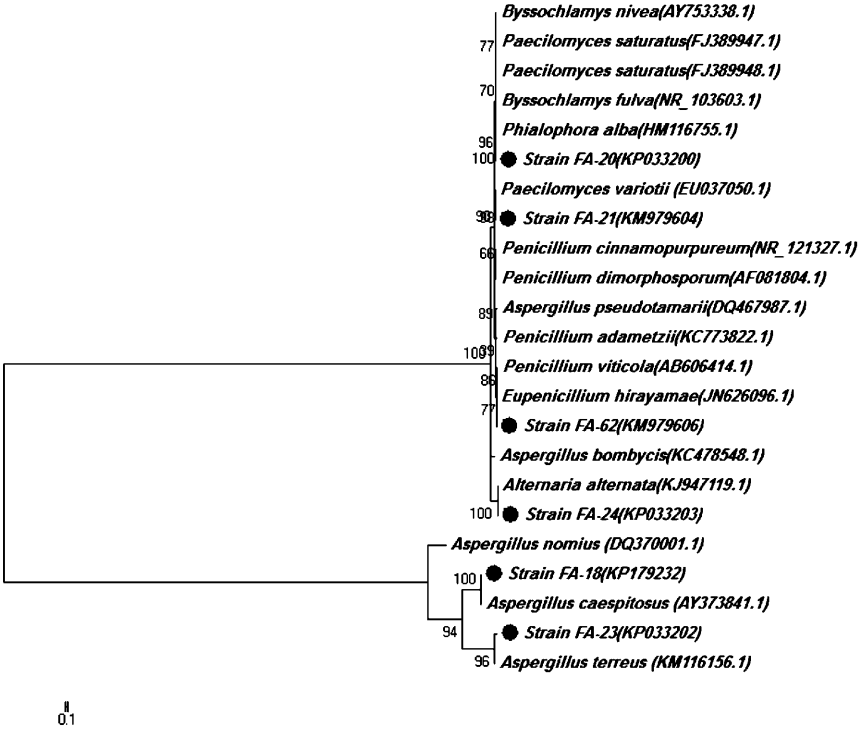

Figure 1. *Phylogenetic tree based on 18S rDNA sequence with maximum likelihood method by using program MEGA 6. Branch support was assessed using 100 bootstrap replicates*

of *Aspergillus caespitosus, Phialophora alba, Alternaria alternata* (Accession No. AY373841.1, HM116755.1, KJ947119.1) respectively and *FA-21, FA-23, FA-62* strains were 99% identical to that of *Paecilomyces variotii*, *Aspergillus terreus*, *Eupenicillium hirayamae* (Accession No. EU037050.1, KM116156.1, JN626096.1) respectively. Molecular analysis of fungal rDNA at the sequence level provides a powerful technique for assessing fungal diversity at the genus level. PCR primers play a crucial role in the molecular assessment of environmental microbes. The specificity of the primer pairs is vital in this context to allow selective or enriching amplifications of fungal rDNA genes from environmental DNA [30].

Fungal Growth on LDPE Film

During the fungal treatment of LDPE, inocula of six isolates gradually developed association with the film and within 15-20 days grew to form colonies of variable size and appearance. These isolates, which were inferred to be active on LDPE, included *Alternaria alternate, Aspergillus flavus*, *A. terreus*, *Emericella nidulans*, *Paecilomyces variotii*, *Penicillium duclauxii* and *P. vinaceum*. In the absence of LDPE film (controls) none of the isolates, including *Alternaria alternata* (**Figure 2A**), showed any appreciable growth of the inocula. On the other hand, as shown in **Figures 2B-D**, profuse growth of *Aspergillus terreus, Penicillium vinaceum,* and *Paecilomyces variotii* respectively was noticed around the film pieces.

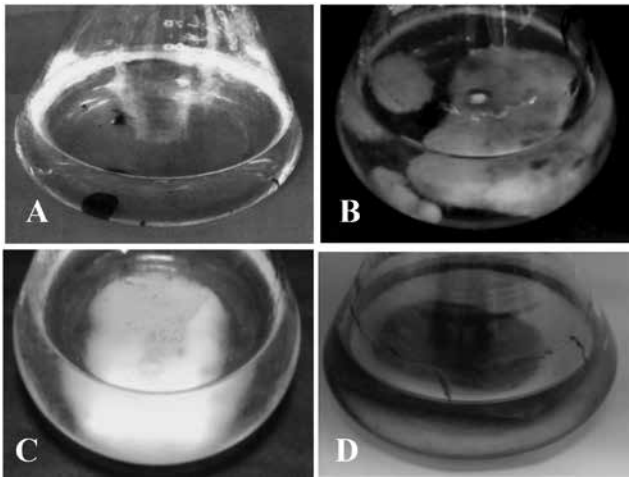

Figure 2*. Growth of fungi with LDPE film: (A) Control (Without LDPE film) Alternaria alternata, showing meagre growth; (B-D) Treatments (with LDPE film) Aspergillus terreus, Eupenicillium hirayamae, and Paecilomyces viriotii respectively, showing profuse growth of fungus in association with LDPE film*

Fungi are considered to be highly potent candidates for biodegradation of plastic materials because of their ability to bind to the surface of the substrate [18, 31, 32] and their capacity to produce enzymes of diverse nature under hugely variable conditions [33, 34]. Our results demonstrate sufficiently that degrading fungi grew around the LDPE film, which may not be possible without a firm attachment between the fungal cells and the substrate surface. Furthermore, profuse growth of the active fungi around the film in the absence of any carbon source in the broth indicates that the fungus has been consuming the film, which can be possible only after needful breakdown of the film material. This inference is further strengthened by almost non-existent growth of inocula under control conditions. Pramila and Ramesh [29] have convincingly demonstrated the attachment of degrading *Aspergillus* species to LDPE film in cultures. Raaman et al. [35], have also shown attachment and growth of *Aspergillus japonicas* cultures on LDPE film resulting in appreciable extent of degradation.

Biomass Accumulation by Fungi Under Co-cultivation With LDPE

Six fungal isolates were found to be growing in association with LDPE film after 4 weeks. These isolates showed greater accumulation of biomass as compared to their corresponding controls (**Table 1**). *Alternaria alternata* gained biomass of 73.4% followed by *Aspergillus terreus* (61.6%); whereas minimum weight gain (38.4%) was recorded in *Paecilomyces variotii*. Consortium of all the isolates under test showed the highest gain in biomass (81.04%) suggesting a synergistic effect of the fungi acting together. Biomass

Table 1. Biomass accumulation by fungal isolates during co-cultivation with LDPE film

Fungal isolate	Dry weight comparison after 4-week co-cultivation*			
	Controls (g/l)	Treatments (g /l)	Gain due to biodegradation	
			Weight (g/l)	(%)
Alternaria alternata	0.642 ± 0.010	1.113±0.007	0.471	73.4
Aspergillus caespitosus	0.462 ± 0.051	0.658 ± 0.003	0.196	42.4
Aspergillus terreus	0.625 ± 0.011	1.010 ± 0.009	0.38	61.6
Eupenicillium hirayamae	0.555 ± 0.010	0.804 ± 0.006	0.249	44.9
Paecilomyces variotii	0.594 ± 0.038	0.847 ± 0.102	0.253	42.6
Phialophora alba	0.524 ± 0.005	0.725 ± 0.005	0.201	38.4
Consortium	0.707±0.116	1.28±0.41	0.573	81.04
**Data represents mean of three replicates ±Standard Deviation*				

accumulation in association with LDPE in the absence of any other source of carbon is an evidence of degradation of the polymer for nutritional purpose [32]. Several other studies, which claimed to demonstrate biodegradation potential of different fungi, have also reported biomass accumulation under similar conditions. Pramila and Ramesh [29] have reported a significant gain in fresh weight of two *Aspergillus* species co-cultivated with LDPE powder. Mahalakshmi and Andrew [36] showed that *Rhizopus* and *Penicillium* species co-cultivated with LDPE pieces treated with a combination of heat and UV irradiation accumulated significantly higher biomass as compared to the controls cultures without the film. Through ergosterol estimation method, Da Luz et al. [37], demonstrated high biomass gain by *Pleurotus ostereatus* cultures on oxo-polymer film indicating biodegradation activity of the fungus.

Esmaeili et al. [16], isolated several fungi and bacteria from landfill soil and identified one isolate each of a fungus (*Aspergillus niger*) and a bacterium (*Lysinibacillus xylanilyticus*) as active on LDPE film. When microbial mixture was grown with soil and LDPE film, microbial count, biomass carbon was found to be higher in treatments as compared to the controls.

Enzyme Activity of Fungal Isolates

Production of laccase, MnP, and LiP enzymes was recorded in most of the control and treatment cultures of the six tested fungi as well as their consortium (**Table 2**). Under control conditions, *Aspergillus flavus* did not produce laccase, *Penicillium vinaceum* lacked MnP, and *Alternaria alternata* and *Paecilomyces variotii* did not show Lip; while *Penicillium duclauxi* was devoid of laccase and LiP. Under treatment, *Alternaria alternata* and *Penicillium duclauxi* did not produce LiP. This variation in enzyme production pattern may be genotypic in nature. Wu et al. [26], investigating biodegradation potential of *Fusarium solani* strains against anthracene (ANT) and benz[a]anthracene (BAA), recorded laccase to be the only active enzyme and found no traces of MnP and LiP. In general, level of MnP was highest among the three enzymes followed by laccase and LiP. Level of the three enzymes was conspicuously higher in cultures growing in association with LDPE film as compared to the controls; probably because, here the enzymes were in greater demand for polymer breakdown reactions. Consortium of the six tested fungi showed remarkably higher level of enzymes as compared to the individual fungi under both, control and treatment conditions. This may be due to synergistic effect of the pooled genotypes. *Alternaria alternate* produced high level of laccase (38.05±1.44 U ml^{-1}) and MnP (53.97±3.20 U ml^{-1}) followed by *Aspergillus terreus* with laccase (22.12±2.21 U ml^{-1}), MnP 19.86±1.98 U ml^{-1}), and LiP (2.1±1.01 U ml^{-1}), which corroborates well with biomass accumulation in the same order (**Table 1**).

Table 2. Enzyme activity of fungal isolates during co-cultivation with LDPE film

Fungal Isolate	Enzyme activity after 4-weak co-cultivation*					
	LAC Uml^{-1}		MnP Uml^{-1}		LiP Uml^{-1}	
	Controls*	Treatments	Controls	Treatments	Controls	Treatments
Alternaria alternata	ND ND ND	2.6±0.84	ND ND ND	53.97±3.2	6.15±1.5	38.05±1.4
Aspergillus caespitosus	ND ND ND	4.3±0.58	2.65±1.1	16.61±1.6	9.98±1.5	3.34±0.82
Aspergillus terreus	1.6±0.61	2.1±1.01	1.56±0.6	19.86±1.9	1.51±0.7	22.12±2.2
Eupenicillium hirayamae	ND ND ND	17.00±0.97	1.78±0.6	40.80±1.0	ND ND ND	ND ND ND
Paecilomyces variotii	ND ND ND	12.68±1.2	2.4± 0.66	20.71±1.5	ND ND ND	4.9±0.26
Phialophora alba	ND ND ND	8.69±1.3	3.92±0.6	19.40±0.6	2.4±0.65	1.5±0.51
Consortium	6.04±1.95	74.70±4.9	20.27±2.9	105.49±6.5	9.8±2.48	37.1±4.20

**Data represents mean of three replicates ±Standard Deviation; ND: Not detected*

Biodegradation of a high mass polymer like LDPE has to be facilitated by extracellular enzymes released by the acting microorganism [38, 32, 17, 39]. Hence, elevated enzyme levels in our treatment broths are direct indication of the degenerative activity. Wu et al. [26], demonstrated high level of laccase in a treatment of LDPE with *Fusarium solani* strains leading to degradation of the polymer. El-Shafei et al. [20], have also reported enhanced enzyme activity of *Mucor rouxii, Aspergillus flavus,* and *Streptomyces* when co-cultivated with polyethylene film.

CO_2 Elution Due to Fungal Activity on LDPE Film

No significant difference was observed between CO_2 evolution estimates taken by volumetric and gravimetric methods; therefore, data was merged as means of the two procedures for each fungal isolate (**Table 3**). *Alternaria alternata* showed a significant enhancement in Co_2 emission over the control (75.9%) followed by *Aspergillus terreus* (63.0%) and *Penicillium duclauxii* (56.2%). Again, Consortium of isolates showed the highest activity reflected in maximum CO_2 liberation (79.7%). This trend closely matches with the trends of biomass accumulation and enzyme activity suggesting that variable degree of biodegradation of LDPE has taken place by fungal activity reflected in corroborative levels of enzyme production, biomass accumulation, and CO_2 liberation.

Table 3. CO_2 evolved during co-cultivation of fungal isolates with LDPE film

Fungal Isolate	Comparison of CO_2 emission after 4-week co-cultivation			
	Control* (g/l)	Treatment (g/l)	Enhancement due to biodegradation	
			Weight (g/l)	(%)
Alternaria alternata	0.460 ± 0.003	0.809 ± 0.008	0.349	75.9
Aspergillus caespitosus	0.397 ± 0.002	0.532 ±0.003	0.135	34.0
Aspergillus terreus	0.435 ± 0.005	0.709 ±0.003	0.274	63.0
Eupenicillium hirayamae	0.445 ± 0.004	0.695 ±004	0.250	56.2
Paecilomyces variotii	0.452 ± 0.006	0.666 ±006	0.214	47.3
Phialophora alba	0.466 ± 0.010	0.647 ±003	0.181	38.8
Consortium	0.497±0.14	0.893±0.13	0.396	79.7
**Data represents mean of three replicates ±Standard Deviation*				

Under aerobic conditions fungi enzymatically breakdown polymer chains of different sizes; the process generates low mass fractions, CO_2, and H_2O_2. Hence, release of CO_2 during co-cultivation of fungi and plastics like LDPE in broth is considered a reliable indicator of biodegradation [32]; and its estimation is considered a standard method for assaying the biodegradation activity [17]. While assessing the biodegradation potential of *Aspergillus niger* isolates against polyethylene granules in soil matrix, Esmaeili et al. [16], noted significantly higher evolution of CO_2 in treatments supplemented with the fungus. Pramila and Ramesh [29], recorded higher emission of CO_2 under treatment of LDPE film with *Aspergillus* species. Shah et al. [40], treated polyethylene bag pieces with *Fusarium* culture isolated from sewage waste and found remarkably higher evolution of CO_2 in the treatments as compared to the controls.

Surface Features of LDPE Film Under Light Microscope

Surface appearance of the LDPE film before treatment and subsequent growth of the fungus on the surface of the film as seen under light microscope (40x) has been depicted in **Figure 3**. Prior to the initiation of treatment LDPE film showed a smooth surface without any stains (**Figure 3A**); whereas after the treatment (**Figures 3B-D**) same film showed profuse growth of *Eupenicillium hirayamae*, *Aspergillus terreus*, and *Phialophora alba* respectively all over

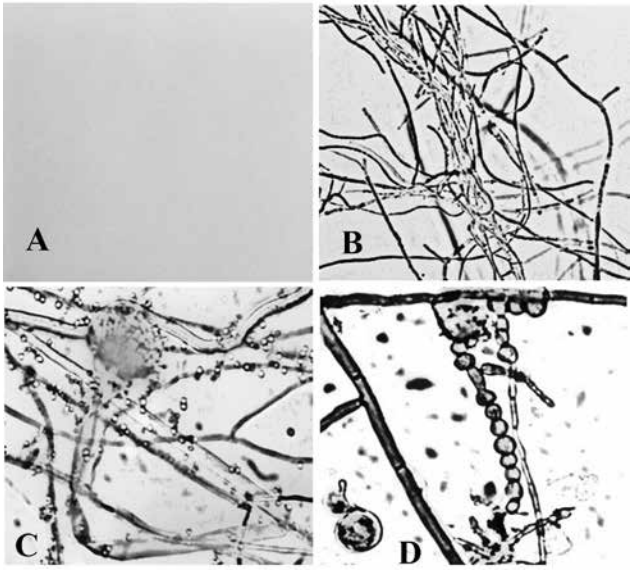

Figure 3. *LDPE film as seen under light microscope (40X): (A) Before treatment; (B-D) After co-cultivation with fungal isolates: Eupenicillium hirayamae, Aspergillus terreus, and Phialophora alba respectively*

the surface. Attachment and growth of fungus on the film indicates that it is feeding on the material of the film and in the process must be degrading it. In concurrence with our observations, Poonam et al. [31], have shown colonization of *Emericella* and *Aspergillus* species on LDPE film and concurrent degradation of the film. Similar microscopic observations of *Aspergillus* species growing on LDPE film have been presented by Raaman et al. [35] and Pramila and Ramesh [29], as well.

Surface Features of LDPE Film Under SEM

Scanning electron micrographs of LDPE film before and after the treatment provided a direct and incontrovertible evidence of fungal activity on the LDPE film (**Figure 4**). Before the treatment, surface of the film appeared absolutely clean and smooth (**Figure 4A**). However, post-treatment micrographs show variable intensity of fungal growth on the films where mycelia and conidia could be seen physically associated with the surface (**Figures 4B-H**). These images depict attachment and growth of *Alternaria alternate, Aspergillus flavus, Aspergillus terreus, Penicillium duclauxii, penicillium vinaceum, Paecilomyces variotii,* and *Emericella nidulans* respectively on the surface of LDPE film. Scanning electron micrographs of *Aspergillus* species of marine origin growing in association with LDPE film have been presented by Pramila and Ramesh [29] also. In the study of Raaman et al. [35], too SEM images of *Aspergillus* species grown on LDPE film were provided; but these fungal genotypes were isolated from solid waste scattered around polluted sites. Sahebnazar et al. [19], have produced SE micrographs displaying disrupted surface of LDPE film and penetration of the hyphae into the film after treatment with *Aspergillus* species in combination with UV irradiation. Using ATCC strains of *Aspergillus niger* and *Penicillium pinophilum* Volke-Sepulveda et al. [18], showed through SEM images, that fungal hyphae virtually coalesce with LDPE film as they grow over the surface. On the basis of FT-IR and X-Ray diffraction analysis, and scanning electron microscopy Esmaeili et al. [16], recorded that LDPE film treated with microbial combination showed extensive changes and erosion in surface characteristics. They have also noticed declined tensile strength of the treated film. Observations under the current study and previous reports strongly suggest that some fungi have the capacity to solubilize the surface matrix of LDPE and utilize the same for their growth.

CONCLUSIONS

This is the first research evaluating the ability of the fungal strains isolated from mangrove species *Avicennia marina* and *Rhizophora mucronata* on the

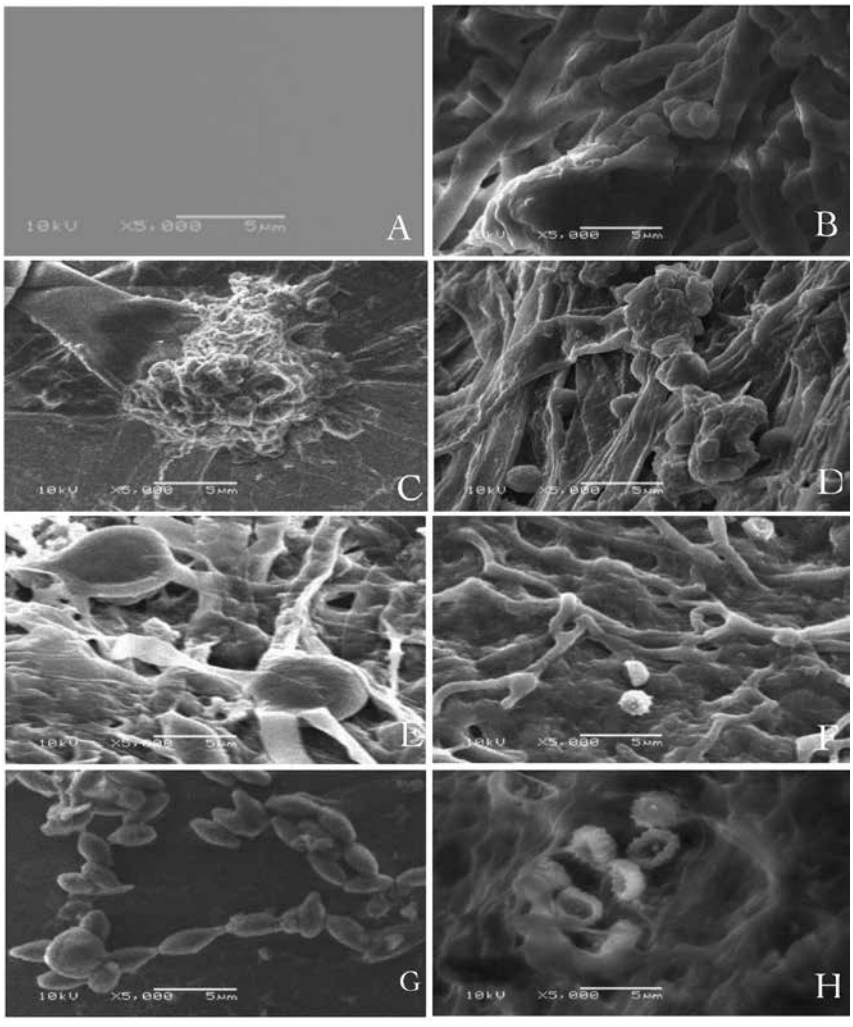

Figure 4. *Scan electron micrographs of treated LDPE film: (A) Before treatment (control) – showing smooth and clean surface; (B-H) After co-cultivation with fungi – Alternaria alternate, Aspergillus caespitosus, A. terreus, Eupenicillium hirayamae, Paecilomyces variotii, Phialophora alba, and Consortium respectively, showing profuse growth of fungal mycelia*

Red Sea Coast of Saudi Arabia to breakdown and consume plastics like LDPE and is the first report documenting the diversity of marine fungi from these locations. It is interesting to note that more biomass, liberation of CO_2 and enzyme production lead to higher LDPE degradation values, confirming that these strains could be interesting to use in future field tests.

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