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Screening of Polyethylene Degrading Fungi from Polyethylene Dump Site

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Abstract : Fungus associated with the polyethylene degradation present in Polyethylene polluted sites, was isolated and identified by serial dilution and staining technique. Various physicochemical parameters of soil sample were tested. The isolated fungus were screened on the minimal salt medium (MSM) supplemented by the polyethylene powder as a carbon source. The growth appearance of the fungi on the polyethylene supplemented minimal salt medium showing the use of polyethylene as a sole carbon source by the isolated fungus. Isolated fungal strains were identified as *Aspergillus niger, A. fumigatous, A. flavus.,A. terrus and Fusarium.* After these antibiotic susptibility and *in-situ* degradation was performed in which these fungus shows high degradabal potential. In all soil sample *Aspergillus niger* was in the most dominating fungal followed by *A. flavus,Fusarium, A. fumigatous, A. terrus* respectively.
Keywords : Polyethylene, Degradation, Fungus, MSM, Physico-chemical parameters, *in situ* degradation.

Introduction

The most commonly used polymer in packaging is Polyethylene. The polyethylene is transformed into packaging material by thermal processes. Every year 25 million tons of synthetic plastics are being accumulated in the sea coasts and terrestrial environment[1]. Plastic products have become an integral part in our daily life as a basic need. It produced on a massive scale worldwide and its production crosses the 150 million tonnes per year globally[2]. These polyethylenes are characteristically inert and resistant to microbial attack and therefore they remain in the nature without any deformation for very long time. When the plastic products mix in the soil and accumulate continuously, they affect the absorption of nutrients and water by the crops, thus reducing their output. Polyethylene is extremely hazardous. They release toxic chemicals which contaminate food item[3]. A very visible portion of municipal and industrial waste consists of polyethylene (PE) films utilized on a massive scale as wrapping material, a typical example for the end consumer growing concern as to whether the plastic litter does not compromises soil quality[4].

Plastic litter has become an omnipresent part of our environment. Although there are almost no data about the environmental fate of the fragments the only known adverse environmental effects of PE films are when they are swallowed by wild animals and encapsulation of material on landfills and in the soil, thus altering microbial processes towards anaerobiosis. For this type of contamination the term ‘‘macro pollutants’’ is sometimes used. The polythene and plastic could sometimes caused blockage in intestine of fish, birds, cow and deer[5]. The drastic rise in the use of non biodegradable plastic materials during the past three decades has not been accompanied by corresponding development procedures for the safe disposal or degradation of these

polymers. It seems that their biodegradation is extremely slow and currently it is hardly possible to make even a rough estimation regarding the time necessary for their biodegradation to some substantial extent[6] but Bioremediation is a simple and environment-safe approach to clean-up water contamination[7].

The microorganisms secrete several enzymes in different quantities which degrade PE, which articulated its degradation efficiency of the microorganism[8]. Fungi were considered favourable for the degradation of PE due to their higher ability to form hydrophobic enzyme proteins, which helped the fungal species in attachment to the polymer surface[9, 10]. Frazer A.C.(1994) concluded that the extra cellular enzymes were responsible for such degradation process. Also, he recorded that these microbes attached to the inert surface of polyethylene with the help of enzymes secreted by them and grow on film by utilizing the LDPE and the polymers are depolymerised and are degraded by the process of mineralization into the carbon dioxide (CO_2), water (H_2O) or methane (CH_4). There is so many other ways from which polyethylene pollution overcome like formation of composit with biodegradable product like Fiber-reinforced polymer[12] Abaca-Glass-banana composites[13, 14]nanopartical mix[15,16] biopolymers formation[17] and biodegradable polymere[18].

The aim of present study was to isolate potent low density polyethylene (LDPE) and high density polyethylene (HDPE) degrading microorganisms from plastic waste dump soil.

Material and method

Sample collection

Soil sample collected from polyethylene dumping site. The soil samples were collected at a depth of 3-5cm, in a sterile container. These samples were collected in sterile polythene bags and tightly packed and carefully transferred to the laboratory for the analysis and stored at 4°C aseptically.

Physico-chemical analysis of soil

Various parameters were tested for the soil morphology, which are pH, Temperature, Moisture content, Alkalinity, Chloride, Organic matter, Phosphorus[19].

Isolation of Polyethylene Degrading Fungus

One gram soil sample was transferred into a conical flask containing 99ml of sterile distilled water. This content was shaken and serially diluted[20] and pour plated in sterile Czapek Dox Agar to isolate heterotrophic fungi. The plates were incubated at 28°C for 4 days.

Preparation of Polyethylene powder

Polyethylene sheets were cut in to small bits and immersed in xylene and boiled for 15 min. xylene dissolve the LDPE and the residue was crushed while it was warm by hand with help of pestle and mortar. The LDPE powder so obtained was washed with ethanol to remove residual xylene and allowed to evaporate (3 h) to remove ethanol. The powder was dried room temperature. The LDPE powder was stored in closed containers in room temperature[21].

Minimal salt medium

Polyethylene powder was added in mineral salt medium containing (g/l of distilled water): NH_4NO_3, 1.0; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1.0; $CaCl_2.2H_2O$, 0.1; KCl, 0.15; and yeast extract, 0.1; and 1.0 mg/l of each of the following micro-elements: $FeSO_4.6H_2O$, $ZnSO_4.7H_2O$ and MnSO4; Polyethylene powder was added in mineral salt medium at a final concentration of 0.1% (w/v) respectively and the mixture was sonicated for 1hour at 120 rpm in shaker. After sonication the medium was sterilized at 121°C and pressure for 15 lbs for 20 minutes. About 15 ml sterilized medium was poured before cooling in each plate.

Screening of Polyethylene degrading microorganisms

The isolated organisms were inoculated on polymer containing agar plates and then incubated at 25-30°C for four weeks[22]. The organisms showing growth on the MSM supplement with polyethylene were selected for further analysis, and measured their colony diameter for specific selection [23].

Identification of fungus

Fungus was identified on the basis of macro and microscopic view. Lacto-phenol cotton blue was used to stain the fungi for microscopic view and on the spore's arrangement[24].

Antibiotic sensitive test

Antibiotic sensitivity of selected fungi was checked by disk diffusion method and measured the inhibition zone[25].

***in situ* degradation of polyethylene by selected fungus**

For the *in situ* degradation of polyethylene soil was artificially contaminated by known amount of polyethylene which was dipped in minimal salt medium with different selected fungi. Measures the weight of LDPE and HDPE before and after incubation, monitor the rate of degradation [26, 27].

Weight loss=initial weight- final weight

%Weight loss = initial weight- final weight/initial weight ×100

Result

Different soil sample was collected from the polyethylene dumping site. Various physico-chemical parameters were tested i.e. pH (6-9), Temperature (25-37), Conductivity (0.2ms-1.53ms), Total alkalinity (30-60 mg/l), Organic Matter (0-1.23%), % Chloride (0.01-0.09%), Moisture Content (11-49%) and Phosphorus (180-290mg/l) (table 1). 15 fugal isolates were isolated from the sample and screened with the polyethylene supplemented medium from which only 13 isolates showed growth on the polyethylene supplemented mineral salt medium while two isolates did not showed any growth. Isolates selected for further study on the basis of colony diameter measurement are given in table 2. Fungal isolate PF10 was found highest colony diameter i.e. 91.44 after that PF 12, PF4, PF13 and PF 8 i.e. 83.82, 78.74, 63.5 and 50.8 respectively in decreasing order. These five isolates were selected for the identification and further study. Organisms were identified based on both microscopic and macroscopic observations and identified as *Fusarium sp., Aspergillus flavous, A. fumigatous, A. nigar*,and *A. terrus* respectively. Antibiotic susptibility was check for every selected fungus for four antifungal discs for the safety purpose or cure if any disease cause by these fungal isolates. Out of four antibiotic nystatin was effective for all the fungal isolates and fluconazole is less effective (table3). *In situ* degradation also performed in Kanya Gurukul Campus Haridwar. By known amount of polyethylene which is given in table 4 where *Fusarium* shows best degradation i.e. 77.668 % for LDPE and 43.285 % for HDPE.

Table I: Physico-chemical parameters of soil sample

Soil sample	pH	Temp. (^{0}C)	Conductivity	T.A (mg/l)	%OM	% chloride	%M.C	Phosphorus (mg/l)
A	8.228	37	60.4 µs	30	0.87	0.01775	13.042	290.4
B	9.480	33	1.53 ms	30	2.26	0.01562	49.073	180.9
C	7.758	35	0.617 ms	40	1.23	0.0923	11.158	185.0
D	6.857	25	0.271 ms	60	0	0.03195	32.344	270.0

Abbreviation: Temp: Temperature, T.A: Total alkalinity, OM: Organic Matter, M.C: Moisture content.

Table II: Measurement of colony diameter for screening

S. No	Isolate Code	Colony diameter (mm)* ± SD
1	PF 1	25±0.2
2	PF 2	12±1.2
3	PF 3	34±1
4	PF 4	**78.74**±0.2
5	PF 5	23.1±0.5
6	PF 6	25.4±0.57
7	PF 7	25.4±0.55
8	PF 8	**50.8**±0.1
9	PF 9	17±0.5
10	PF 10	**91.44**±0.6
11	PF 11	34±0.5
12	PF 12	**83.82**±0.2
13	PF 13	**63.5**±0.1

*Average of triplicates

Table III: Antibiotic sensitivity test by disc diffusion method

S.no	Isolates	Antibiotics sensitivity by clear zone method (mm) (average of triplicates)			
		Amphotericin (AP)	**Nystatin (NS)**	**Ketoconazole (KT)**	**Fluconazole (FLC)**
1	***A. Niger***	19.33±0.57	19±1	9±1	-
2	***A. Flavous***	14±0.57	19.67±0.57	18.67±1	-
3	***A. fumigatous***	15±0.57	17±1	15±1	-
4	***A. terrus***	-	14±0.57	18.33±0.57	-
5	***Fusarium solani***	-	11.33±0.57	-	-

Table IV:-*in situ* polyethylene degradation

S.no.	Isolates	Type of polyethylene	Dry weight of polyethylene (gm) (average of triplicates)			
			Initial weight	**Weight after degradation**	**Final degraded weight**	**% Degradation**
1	***A. niger***	LDPE	0.641	0.595	0.046	7.176
		HDPE	0.910	0.907	0.003	0.329
2	***A. Flavous***	LDPE	0.921	0.808	0.113	12.269
		HDPE	0.705	0.682	0.023	3.26
3	***A. Fumigatous***	LDPE	0.594	0.443	0.151	25.42
		HDPE	0.566	0.562	0.004	0.706
4	***A. terrus***	LDPE	0.509	0.448	0.061	11.98
		HDPE	0.525	0.514	0.011	2.095
5	***Fusarium solani***	LDPE	1.630	0.364	1.266	**77.668**
		HDPE	0.700	0.670	0.303	**43.285**
6	Control	LDPE	0.708	0.703	0.005	0.706
		HDPE	1.161	1.159	0.002	0.172

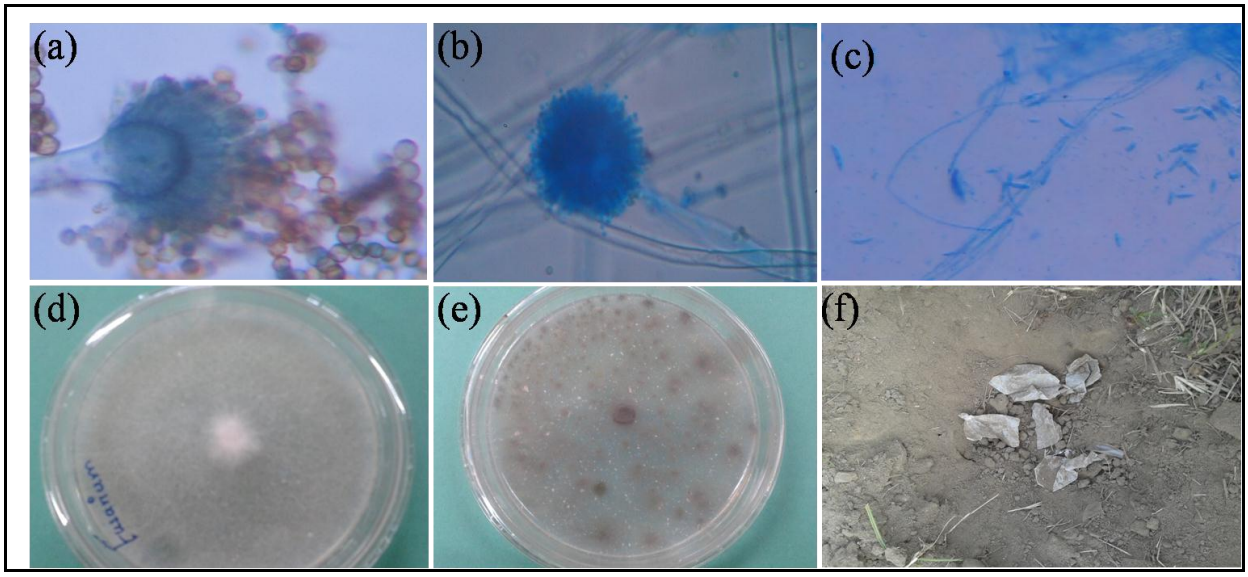

Fig 1 (a)*Aspergillus flavous*(b)*A.niger*(c)*Fusarium* (d and e) Growth on MSM (colony diameter) (f)*in situ* degradation

Discussion

Fungi were isolated from polyethylene dump site soil of Hardwar district. Organism were identified and screened by measurement of colony diameter on minimal salt medium containing polyethylene as a sole carbon source. Sowmaya et al. (2012) mentioned that colony diameter of *A. flavous* about 8 mm Ibrahim et al, (2011) also perform colony diameter screening of fungal species for *A. flavous* (80mm), *A. fumigatous* (30mm), *A. terrus* (80 mm), *Fusarium* (80 mm) but in our study we find larger colony diameter i.e 83.82 mm for *A. flavous* and for *Fusarium sp*., *Aspergillus fumigatous*, *A. nigar* , and *A. terrus* colony diameter is 91.44, 78.74, 63.5 and 50.8 respectively which is higher than above study so these isolates is more efficient for the polyethylene degradation.

Conclusion

From the above work conclude that *Fusariumsp*.,having the highest potential to grow on the polyethylene supplemented medium and high degrading capacity of HDPE and LDPE as compare to other isolates of *Aspergillus* spp so it can be assume that these indigenous fungi can be isolated from polyethylene polluted site by traditional technique e.g., serial dilution and these isolates may be degrader of polyethylene. These fungus isolates are sensitive for various antibiotics which mean these funguses are curable also. So these species of fungus can be use for the *ex situ* and *in situ* degradation of polyethylene in future.

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