Aspergillus nidulans as a fungi that grows on low density polyethylene

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Abstract:

Polythene is known for their easy to use properties due to which they are widely being used up all around the world. Low density polythene is one such type which is flowing in the market in and around on a daily basis. These polyethylene causes harm to the environment as these cannot be degraded easily and takes long years to get degraded. Microorganisms are known for their biodegradation ability, as they are natural scavengers. They are the base of food chain where they do the work of biodegradation. Fungi specifically are such microorganisms having excellent biodegradation ability. In this study, an attempt has been made to isolate such fungal species which can help in biodegradation of LDPE.

Keywords: Fungi, LDPE, Biodegradation, *Aspergillus* sps.

Introduction:

Solid waste are tough to manage, especially they fall in the category of plastics i.e. polythene. Plastics or polythene are well known for their easy handling and usefulness. They make a big part of the solid waste that gets dumped into the environment every day. Solid waste when biodegradable can still be managed but when it is non-biodegradable, it creates a problem. Polythene are said to be non-biodegradable as they cannot be easily degraded. Mainly two types of polyethylene are produced- HDPE and LDPE. HDPE are high density polyethylene produced under low pressure and are usually recycled, so it does not go into the environment directly. These are since recyclable are not harmful ones. These are available as thick polythene bags that are obtained in big shops and shopping malls. LDPE is low density polyethylene produced under high pressure. Though these are recyclable, these are not recycled properly and after a single use goes directly in to environment as a waste product. These are the flimsy polythene bags that are available with small vendors like vegetable vendors, laundry bags etc. These when enter in the environment, either gets burned in the landfill producing harmful gases or reaches the land and water where unknowingly are being eaten up by the animals, leading to their unfortunate death. So a way has to be found out by which LDPE can be removed from the environment safely.

Microorganisms as mentioned before have great biodegradation ability. Bacteria, Fungi, actinomycetes etc. are such examples. Fungi are very much known for their antibiotic activity which points towards the enzymes that they produce. They are producers of enzymes based on the substrate on which they grow. They can grow very easily anywhere may it be land, water or soil. They utilize the substrate as energy source releasing CO2 and water in the environment, which is not harmful. Whether or not Fungi can utilize LDPE as substrate is experimented in this study.

Material and Method:

Collection of Soil Sample:

Soil sample was collected in clean sterilized containers with the help of cork borer and transferred in the containers. It was collected from wet area of Pirana landfill in Ahmedabad. The collected sample was taken to the laboratory and stored for further experimental purpose.

Collection of LDPE:

New and fresh LDPE sample was obtained from a polythene manufacturing company called Diti Enterprise in Vatva GIDC, Ahmedabad.

Preparation of LDPE Powder:

LDPE powder was prepared by dissolving it in xylene solution. The LDPE sheet was cut into small pieces and immersed in xylene and boiled for 15 to 20 minutes. This is then crushed with hands with the help of band gloves. LDPE powder so obtained is washed with ethanol and dried overnight in hot air oven at 60^{o}C.The dry powder so obtained is stored at room temperature for further use.

Isolation of LDPE degrading Fungi:

A synthetic medium containing following constituents were prepared in 500 ml of distilled water: K_2HPO_4 0.5 g, KH_2PO_4 0.1g, NaCl 0.5 g,$CaCl_2.2H_2O$ 0.001 g, $(NH_4)_2SO_4$ 0.5 g, $MgSO_4.7H_2O$ 0.25 g, $CuSO_4.5H_2O$ 0.001 g, $ZnSO_4.7H_2O$ 0.001 g, $MnSO_4.H2O$ 0.001 g, $FeSO_4.7H_2O$ 0.01 g. 50 mg of LDPE powder was added to it. This LDPE powder is the only carbon source for the fungi that might grow. Soil sample was then serially diluted and 10^{-4} dilution was used to spread on all the petriplates containing synthetic medium. Observation was done after a period of 1 to 2 weeks.

Colonization study on LDPE sheets:

Similar attempt was made with LDPE Pellets also. LDPE pellets of same size and weight were sterilized with ethanol for 30 minutes and then washed with distilled water for 10 minutes. Synthetic medium was poured in petriplates. In each petriplates three LDPE pellets were added and inoculated with 10^{-4} dilution of soil sample. The petriplates were kept at incubation at room temperature for a period of one month and results were obtained then.

Identification of Fungi:

Identification of the fungal strain was done in the laboratory by macroscopic and microscopic examination by using Lacto phenol cottonblue (LCB) staining. The key of Raper and Fennel was followed. Apart from laboratory identification, molecular identification of the fungi was also done with the help of SLS research pvt Ltd, Surat.

Molecular identification experimental Method:

DNA was isolated from the culture, whose quality was evaluated on 1.0% Agarose gel, which provided a single band of high molecular weight DNA. The fragment of gene was amplified by PCR. As ingle discrete PCR band was observed when resolved on Agarose Gel. Then the PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with ITS1 primer using BDT v3.1 Cycle sequencing kit on ABI3730xl Genetic analyzer. The gene sequence was used to

carry out BLAST with the database of NCBI Genbank Database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software programs.

Results and discussion:

The study here deals with the isolation ad identification of such fungal species which can help in biodegradation of LDPE. For this two forms of LDPE- powder and Pellet were taken in to consideration to observe that whether the fungal species grows on both the surface of LDPE. From all the five samples collected from the area, recurringly one fungal species was seen growing in contact with the LDPE sheets.

From the macroscopic and microscopic analysis, the fungal species labeled F2 was identified as *Aspergillus nidulans*. Also the sequencing reports obtained from molecular identification revealed the fungal sample's similiarity with Aspergillus nidulans.

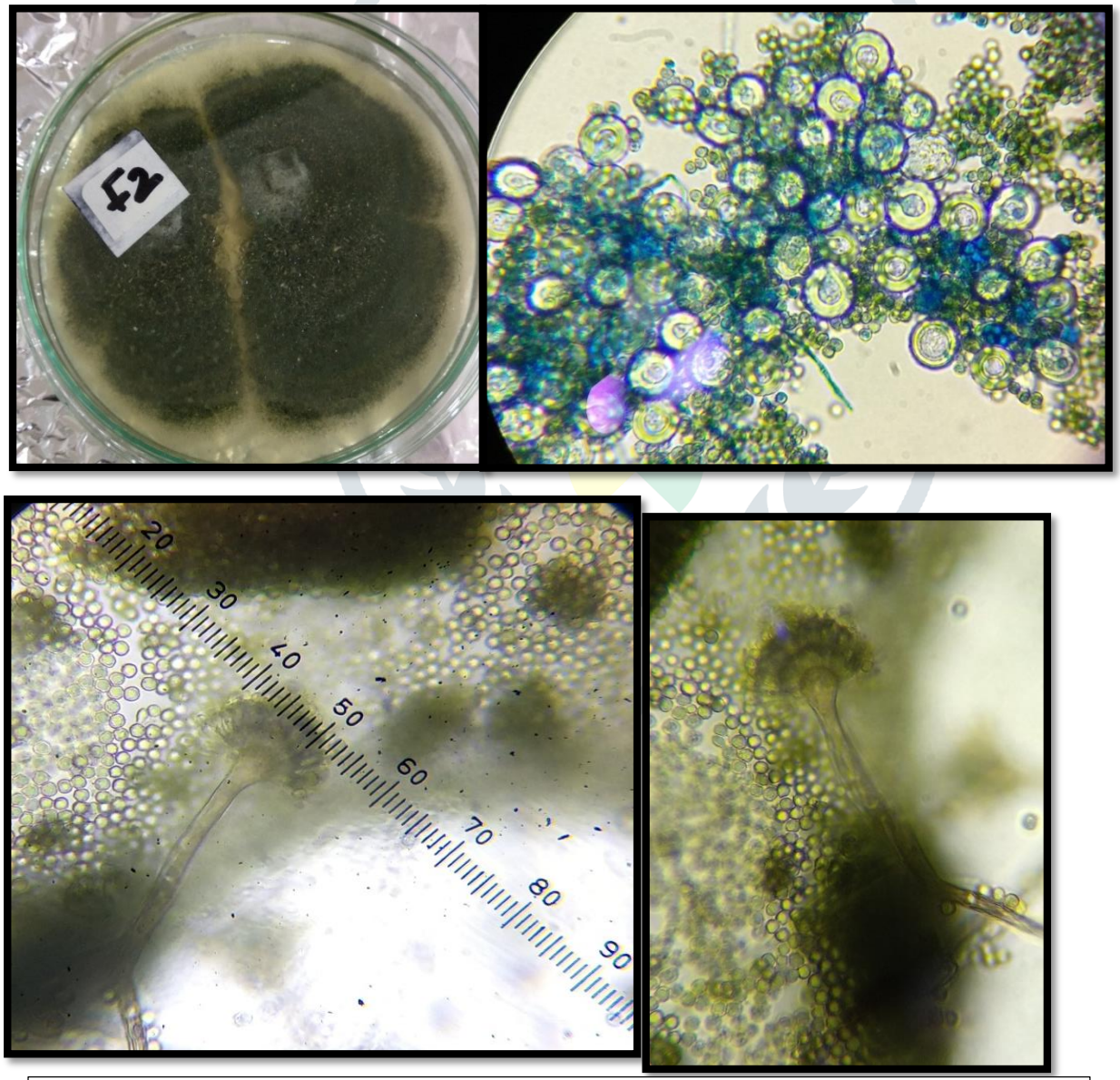

Fig 1: Showing photographs of identified *Aspergillus nidulans*: A) Colony morphology B) Hulle cells C and D) Sporangium of *Aspergillus nidulans*

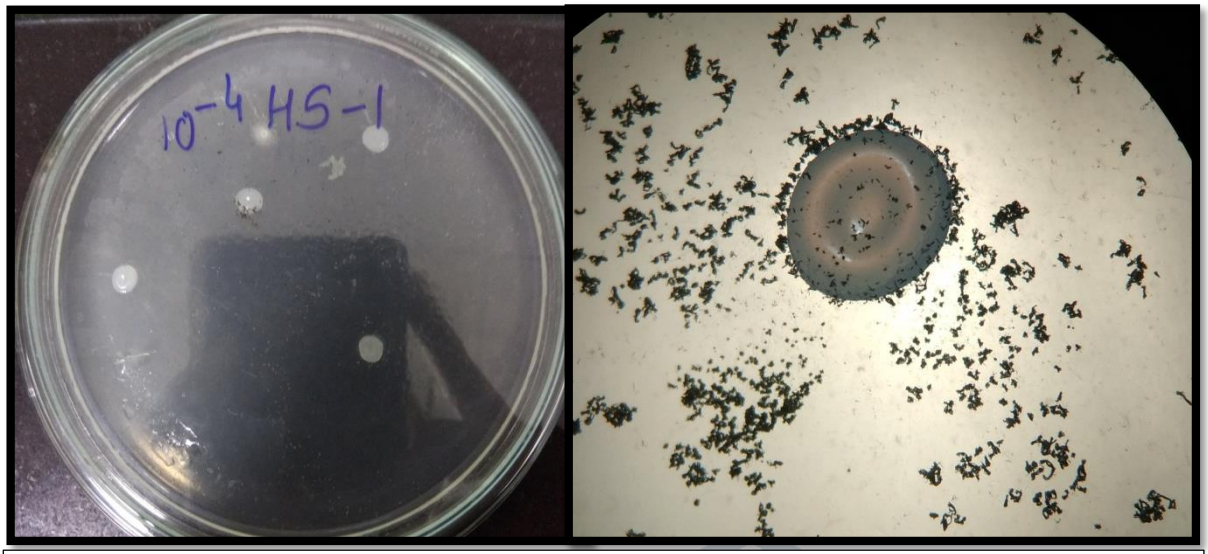

Fig 2:showing photographs of LDPE pellet adhered and surrounded by colony of *Aspergillus nidulans* A) a pellet showing growth of the fungi in petriplate, B) LDPE pellet surface adhered by *Aspergillus nidulans*

Table 1: Sequences producing significant alignments.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Aspergillus nidulans voucher SCFUN3014 internal transcribed spacer 1, partial sequence	385	385	98.00%	6E-103	88.18%	MG780387.1
Aspergillus sp. isolate RS-Apr-ITSC2 small subunit ribosomal RNA gene, partial sequence	379	379	98.00%	3E-101	88.57%	KY096672.1
Aspergillus sp. HT 113 internal transcribed spacer 1, partial Sequence	368	368	98.00%	6E-98	87.94%	KF039712.1
Aspergillus sydowii isolate DFFSCS007 internal transcribed spacer 1, partial sequence	368	368	98.00%	6E-98	87.94%	JX156353.1
Aspergillus ustus isolate Asp-2912 internal transcribed spacer 1, partial sequence	320	320	85.00%	2E-83	87.96%	KY203998.1
Aspergillus ustus strain AP3 internal transcribed spacer 1, partial sequence	320	320	78.00%	2E-83	89.96%	KF860885.1
Fungal endophyte isolate SE4 18S ribosomal RNA gene, partial Sequence	320	320	78.00%	2E-83	89.96%	JQ340077.1
Endophytic Fungus isolate MIB02 18S ribosomal RNA gene, partial sequence	305	305	90.00%	5E-79	86.06%	JN030355.1
Aspergillus ustus strain Jazinak2 internal transcribed spacer 1, partial sequence	270	270	78.00%	2E-68	86.35%	MG890275.1
Aspergillus sp. BAB-4068 18S ribosomal RNA gene, partial Sequence	268	268	81.00%	7E-68	85.50%	KM401400.1

The table shown here gives significant alignment data with different strains of fungi with which the sequence of query i.e. fungal specimen matched to certain percentage. But the highest similiarity was observed with

Aspergillus nidulans voucher SCFUN3014 internal transcribed spacer 1, partial sequence with 98% query cover and an E value of 6E-103, which depicts highest similiarity in comparison to other strains.

Below is given the sequence alignment of query, the specimen of our interest with the strain *ASpergillus nidulans* voucher SCFUN3014 internal transcribed spacer 1, with which highest similiarity was observed. The above molecular identification data proves the fungal species involved in LDPE biodegradation is *Aspergillus nidulans*.

Alignment with most coordinated sequence:

Aspergillus nidulans voucher SCFUN3014 internal transcribed spacer 1, partial sequence

Sequence ID: MG780387.1 Length: 357 Number of Matches: 1

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Query  5    CCGCCGGGGACCACTGAACTTCCCGCCTGACAGTGATGCGGTCTGACCCTGAATACCAAT  64
            ||||||||||||||||||||| ||||||| |||||||| |||||| ||||||||| |||
Sbjct  45   CCGCCGGGGACCACTGAACTTCATGCCTGAGAGTGATGCAGTCTGAGCCTGAATACAAAT  104

Query  65   CACTCAAAACTTTCAAAAATGAATCTCTTGGTTCCGGCTTCGATGAAGAACGCCCCGAAC  124
            || |||||||||||| |||| |||||||||||||| || |||||||||| |||||
Sbjct  105  CAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCNTCNATGAAGAACGCAGCGAAC  164

Query  125  TCCGAAAAGTAATGTGAATTGCAAAATTCATTGAATCATTCTCTCTTTGAACACACATTG  184
            | ||| |||||||||||||||| ||||||| ||||||||    ||||||||| |||||||
Sbjct  165  TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTG  224

Query  185  CGCCCCTTGGCATTCCGGGGGGGCATGCCTGTCCAAACGTCATTGCTGCCTTCAAGCCCGG  244
            |||||| |||||||||||||||||||||||| | |||||||||||| ||||||||||
Sbjct  225  CGCCCCCTGGCATTCCGGGGGGGCATGCCTGTCCNAGCGTCATTGCTGCCCTCAAGCCCGG  284

Query  245  TTTGTGTGTTGGGTCGTCTTccccccGGGGGACGGGCCCTAAAGGAAGGGGCGGCT-CG  303
             ||||||| |||||| | ||||||| |||||||||||| ||||| || |||||| ||
Sbjct  285  CTTGTGTGNNGGGTCGNCGTCCCCCCNGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCG  344

Query  304  TGTCCGGTCCTCG  316
            |||||||||||||
Sbjct  345  TGTCCGGTCCTCG  357
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Table 2: Table shouwing weight loss of LDPE Pellets in an incubation period of a month:

Name of species	weight of the LDPE Pellets				
Aspergillus nidulans	Before incubation	After 7 days	After 14 days	After 21 days	After 28 days
	31.9	30.6	28.9	28.4	26.8

The above results unleash the ability of *Aspergillus nidulans* to grow on LDPE as a substrate. The experiment reveals that *Aspergillus nidulans* utilizes LDPE as carbon and energy source for its survival. This gives an insight that this fungal species might help in LDPE biodegradation though the end product of the reaction is yet to be studied. This fungal species might help in biodegrading LDPE and thus eradicating it from the environment.

Conclusion:

Polythene are harmful to the environment and any attempt to biodegrade it by microbial means will of significance importance. This study shows that fungal species can grow on LDPE utilizing it. It gives way to further research in these lines where more study can be done on its end product formation. If biological way can be used to biodegrade such polythene waste, it will be helpful to the future environment.

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