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ORIGINAL ARTICLE

Biodegradation activity on HDPE plastic (Polypropylene) isolated from marine debris by using aquatic fungus isolated from east coast of Tamil Nadu, India

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ABSTRACT
This present study shows the plastic degradation activity by aquatic fungi isolated from different zones of east coast of Tamil Nadu, India. The research methodologies were conducted to degrade microplastics by using biological factors, especially microorganisms. Herein, fungi isolated from different parts of the east coast of Tamil Nadu, India were tested to check its biodegradable properties to degrade the plastic. The response of fungi biodegradable properties to degrade plastics in a minimum growth medium, was evaluated, based on the quantified mass difference between microplastic discs and fungi used. The ultra-molecular differences were evaluated by using Scanning Electron Microscope (SEM) and the synthetic polymer structure of the plastic was identified by using Fourier transform Infrared Spectroscopy. To identify the fungus, lactophenol cotton blue staining was conducted. Under the tested conditions, the fungal isolates from the east coast of Tamil Nadu, India were able to degrade the plastic, resulting in decrease of mass and molecular changes in the microplastics discs. The research finding show that the marine fungi which are naturally occurring has biodegrade ability to degrade the synthetic polymers under very minimal nutrient conditions in a short span of time.
Keywords: Microplastic, Fungal isolates, degradation

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INTRODUCTION

Microplastics are major pollutants in the aquatic environment. Production and consumption of plastics have continued to increase day by day, especially in the Indian economy [1]. The number of plastics in the marine environment has increased since the development of modern plastics in the early 1900s and their mass production in the 1940s. In India, an increase in the development of Science and Technology, has led to the formation of many synthetic polymer production industries [2]. Due to its low cost, the production rate of plastic is higher but at the same time, dangerous and toxic qualities are ignored. In fact, the pandemic of 2020 has only made use of huge numbers of plastics. The Pollution Control Board (CPCB) report (2018-2019) reports India at a humongous 3.3 million metric tons per year. The biodegradable properties of plastic are based on its physical properties including its crystallinity, orientation, morphology and surface area. It has been revealed that the high diversity of microorganisms in the mangrove soil is capable of degrading plastics [3-6]. Fungi play a vital role in all the ecosystems, including marine ecosystems as decomposers and symbionts owning to their robust morphology and their metabolic activity, they have the ability to biodegrade tough materials like plastics. Fungi have degradable capacity due to the presence of extracellular and intracellular enzyme systems for biodegradation. Four different sites were chosen for isolation of pure strains of fungi and to study the biodegradable properties of synthetic plastic degradation [7-9]. This experiment aims to analyze the potential of fungi to degrade synthetic plastics which are very harmful to the environment and marine ecosystem. The biodegradability of synthetic plastic by using microbes includes attachment of microorganisms to the polymer surface, the growth of microorganisms by depending on the plastic as a nutrient source, i.e., a

carbon source. The plastic polymer degradation is further analysed by mechanical, optical or electrical characteristics, cracking, fission, corrosion, discoloration, phase separation, chemical transformations and formation of new functional groups after the degradation process [10].

MATERIAL AND METHODS

Collection of Fungi samples

The samples were collected from different locations having different environmental conditions, including salt-pan, sewage waste, estuary, and industrial waste.

Salt-pan: Sediment samples were collected from the salt-pans having rich sources of seaweed with a halophilic nature located in Marakkanam, Tamilnadu, India (Lattitude: 12.235549/ longitude: 79.936059).

Sewage waste: Sludge samples were collected from sewage waste having rich mangrove diversity in Cuddalore, old town, Tamilnadu, India. (Latitude:11.712949/ longitude: 79.769438).

Estuary: Sediment samples were collected from a mangrove rich region at vellar estuary, Parangipettai, Tamilnadu, India (Latitude:10.06'E/longitude: 79.27'N).

Industrial waste: Sludge samples were collected from residual waste of industrial regions were waste were mixed with sea water in Cuddalore, Tamilnadu, India. (Latitude: 11.6376 N / 79.73480 E).

Isolation of fungi from the soil samples by serial dilution method and spread plate method

Fungal growth medium

The samples from the locations were collected and serially diluted under various dilution factors for isolation of fungal isolates. The different agar medium was used for culturing fungus. The spread plate technique was performed in the agar medium included using Potato dextrose agar, Sabouraud dextrose agar, Rose Bengal agar was prepared by using autoclaved sterilized 50% estuarine water, 20% sea water and 30% distilled water along with addition of 0.2% of chloramphenicol. 3% of the sediments and sludges collected were also sterilized and autoclaved to provide additional nutrients for the fungal growth. The plates were then incubated under optimum conditions at 25-27^{o}C for 72-120 hours. Then the fungal growth was observed and pure culture technique was carried out by using the same agar formulation for pure fungal isolates under a sterile environment.

Preparation of microplastic discs

The plastic was isolated from the floating debris of velar estuary, Tamilnadu, India. A plastic bag was isolated from the Velar estuary. It was then washed with fresh water and then kept under ultra violet radiation for 30 minutes. The plastic bag was then cut below >5 mm [Fig: 1] using a punching machine. The microplastic discs were then used for biodegradation analysis.

Figure 1: Plastic bag was cut into microplastics with diameter less than >5mm.

Inoculation of plastic discs along with fungal isolates

Broth cultures or liquid cultures are used to grow microbes in laboratories. There were different medium prepared including potato dextrose broth, sabouraud dextrose broth, rose Bengal broth formulated with addition of sterilized 50% estuarine water, 20% sea water and 30% distilled water along with addition of 0.2% of chloramphenicol. Then 3% of the sediments and sludges were formulated into liquid form and added to the broth. The medium is then inoculated with pure strains of fungi and 20% of microplastic discs. It was then kept at optimum temperature under sterilized conditions at 25-27^{o}C for 72-150 hours. Along with that control was also prepared by inoculating the plastics discs in the broth media without any fungal strains. Then it was transferred to a shaking incubator and during of intervals the degradation activity were evaluated.

RESULTS

After 7th day and 30th day incubation, microplastics were taken out from the broth and checked for any biodegradation activity in plastic discs. The change in the structure of the microplastics were calculated based on microscopic SEM images for any cracks, holes, dents in the structure, identification of the polymer compound by FTIR, identification of the fungal isolated by lactophenol cotton blue (LPCB) staining.

Scanning electron microscopy images on 7th day and 30th day analysis:

The images of microplastic discs were taken on 7th day and 30th day to check whether there is any change in the structure like holes, dents, cracks on the plastic by using scanning electroscope microscopy. The controls were also taken and analyzed under the SEM.

Figure 2: The control images of plastic discs on 7th and 30th day under SEM analysis. No change like cracks, dents, holes were observed under SEM.

SEM images on 7th day analysis of microplastic discs

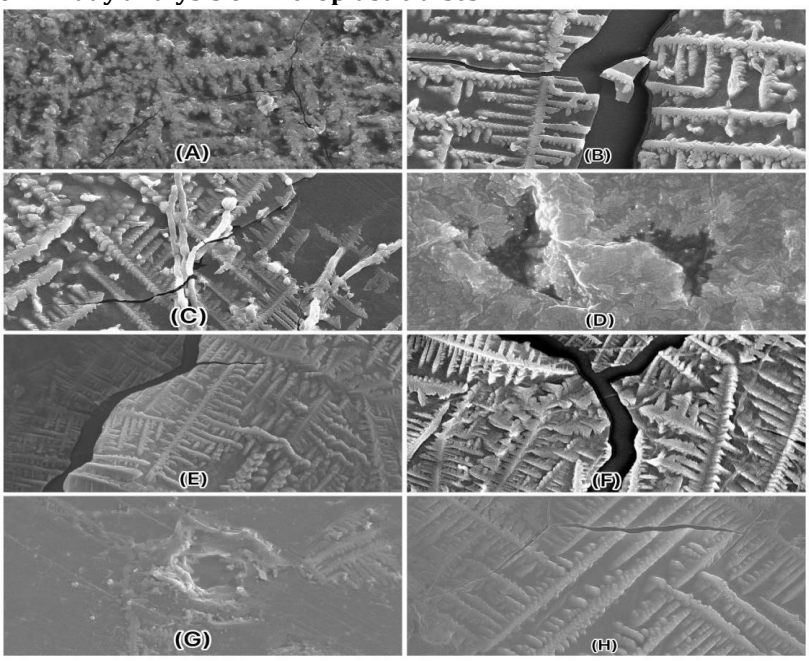


Figure 3: The SEM images were taken after incubation period of 7 days of microplastic discs along with the microplastics. The growth of fungal mycelium was observed under the SEM analysis. In the images A,B,C,D,E.F,G,H indicates that the fungal growth on the plastic discs induces the microlevel changes in the structure of the plastics like casuing dents, holes, cracks.

SEM images on 30th day analysis of microplastic discs

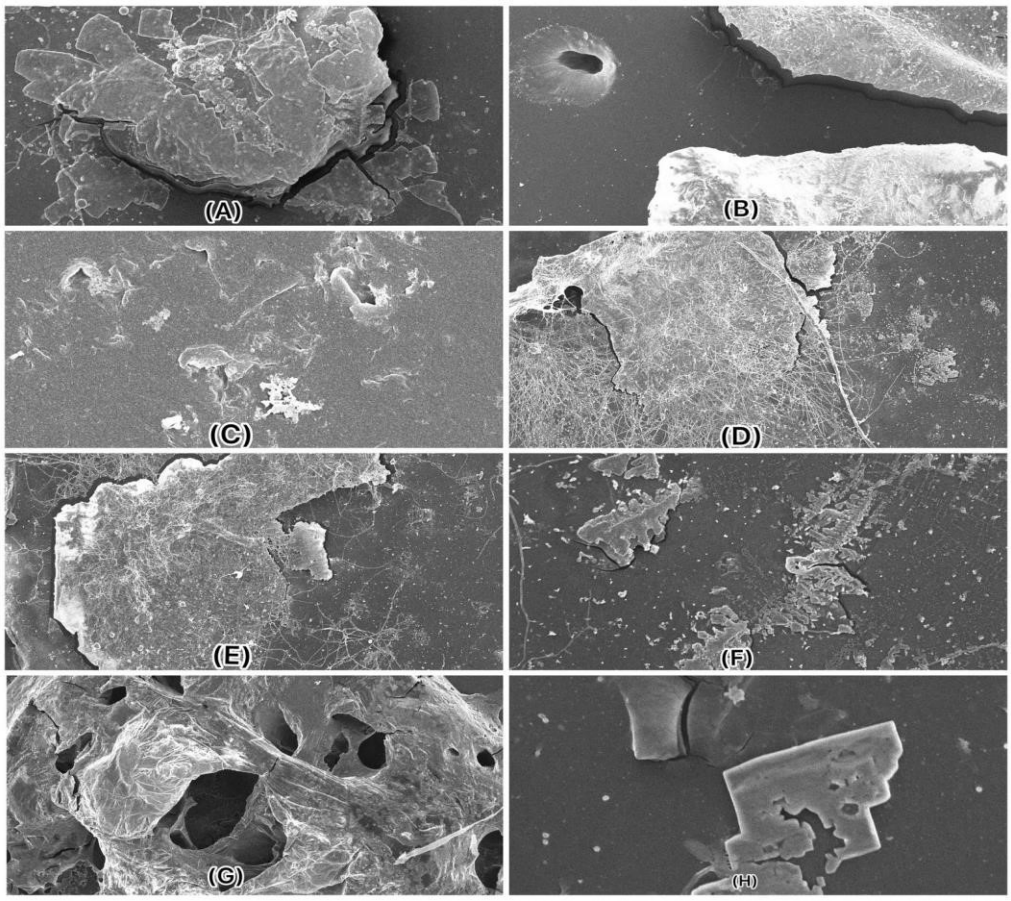


Figure 4: The SEM images were taken after incubation period of 30 days of microplastic discs along with the microplastics. The growth of fungal mycelium was observed under the SEM analysis. In the images A,B,C,D,E.F,G,H indicates that the aquatic fungus was able to show degradation activity like massive micro level changes when compared to 7th day analysis on the plastic discs indicating biodegradation of plastics**.**

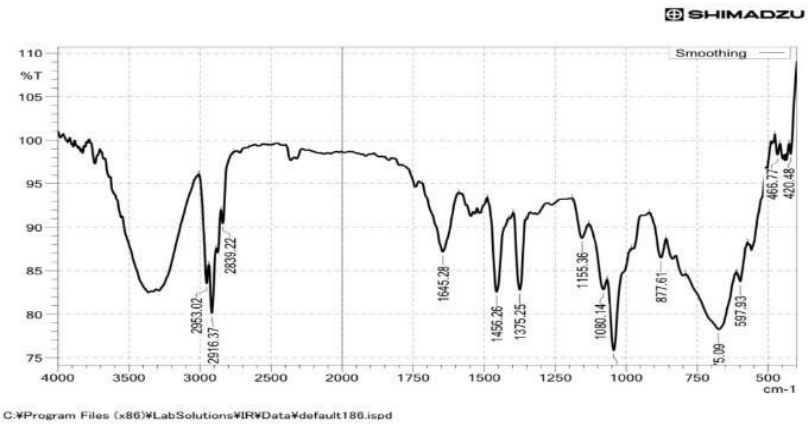
The SEM analysis results from the 7th day and 30th day of microplastics shows that the particular fungal isolated were able to show degradation activity from the group of pure strains tested from each regions. Especially some strains didn't show any changes in the microplastic surface where only five strains able to show degradation of the plastic surface. The most efficient fungal strains isolated from the salt pan and sewage sludge samples were able to show more changes in the plastic structure under scanning electron microscope when compared to industrial waste residues and estuarine mangrove soil. This shows fungal evolves in these regions has the ability to use plastic as a part of their food source. Especially these fungal isolates were able to show degrading activity HDPE plastic such as polypropylene which pollutes the marine environment.

By using the wavelength peaks by infrared rays is used to identify the type of synthetic polymer used for making the plastic bag found in the estuary under FTIR analysis. The results for FTIR spectrum show numerous small peaks in the wave number range 1400-750 cm^{-1}, Bands between 1300 cm^{-1} and 750 cm^{-1} refer to carbon lattice pulsation and the wagging vibration of CH_2-CH, CH_3, and deformation vibrations at 1170 cm-1 & 1150 cm3 indicates CH_2 and CH. The bands present in the spectrum confirm that the plastic isolated from the marine debris is made up of synthetic polymer polypropylene [3, 4].

Lactophenol cotton blue staining (LPCB) for identifying the fungi isolated having degrading properties to degrade polypropylene

The isolated pure strains of fungi were identified by using lactophenol cotton blue staining and viewed under a microscope. Based on its macro and micro structure the fungi strain has been identified. Figure 5: Lactophenol cotton blue staining of the fungal isolates which has excellent degradable properties. Based on its in microscopic morphology the fungal isolates were identified as (A) *Aspergillus sp.,* (B) *Rhizopus sp.,* (C) *Cladospora sp.,* (D) *Penicillium sp.,* (E) *Trichoderma sp.,*

Fourier-transform infrared spectroscopy (FT-IR) for the identification of the polymer



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	420.48	98.42	3.21	426.27	399.26	72.835	43.917	
2	466.77	98.34	1.72	476.42	459.06	16.301	18.318	
3	597.93	83.74	2.28	611.43	572.86	555.983	36.737	
4	675.09	78.26	6.49	790.81	611.43	3375.853	635.150	
5	877.61	86.49	3.10	918.12	860.25	648.933	81.446	
6	1043.49	75.82	9.12	1066.64	979.84	1511.025	277.610	
7	1080.14	82.84	2.45	1132.21	1066.64	891.714	50.358	
8	1155.36	88.74	2.80	1190.08	1132.21	551.536	78.905	
9	1375.25	82.79	8.48	1398.39	1354.03	584.319	198.099	
10	1456.26	82.56	9.81	1489.05	1413.82	886.135	300.473	
11	1645.28	87.19	7.26	1718.58	1589.34	1169.427	461.421	
12	2839.22	90.44	2.53	2852.72	2760.14	388.205	43.315	
13	2916.37	80.12	6.35	2937.59	2885.51	851.126	155.315	
14	2953.02	83.53	4.41	3008.95	2937.59	745.317	95.589	

Figure 5: FT-IR analysis of the plastic bag found in the estuary debris.

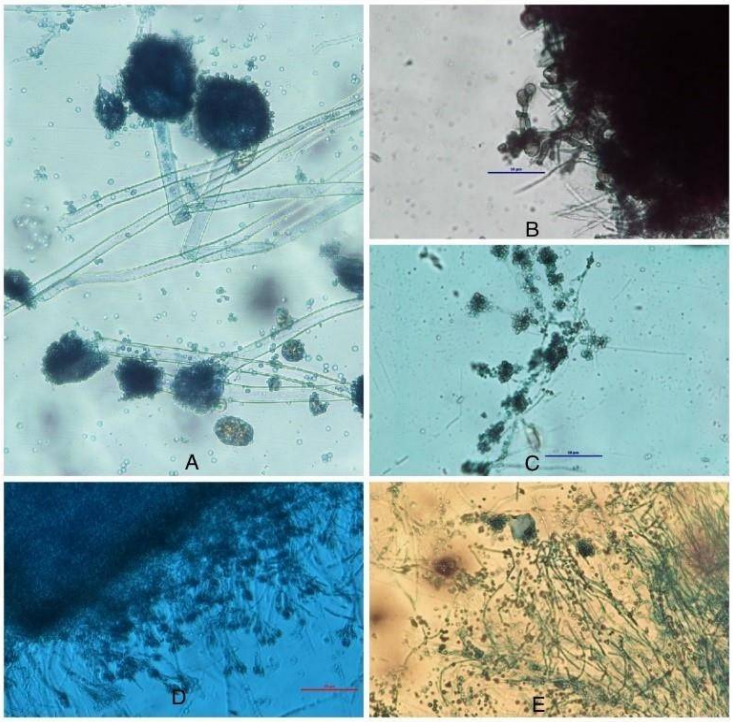


Figure 5: LPCB images of five efficient fungal strains that were able to degrade HDPE plastic polypropylene isolated from the all the four locations.

DISCUSSION

Microplastics made out of synthetic polymers which are not easily biodegradable pollute the environment. The production of plastics has gone higher in the last half of the century. To degrade the synthetic polymer, we have to break its long chain polymer structure. Microorganisms play a vital role in the degradation of synthetic plastics. Most microorganisms, including bacteria, have the ability to degrade

microplastics made out of synthetic plastics. Fungi is more efficient in degradation of synthetic polymer isolated from the marine environment. The fungal colonization and biodegradation of plasticized polyvinyl chloride in in situ and ex situ conditions and suggested that microbial succession may occur during the long periods of exposure in in situ conditions [14]. Here the present study shows that Polypropylene (PP) can also be degraded by fungi more efficiently in 14 days of incubation. Webb et al. [15] also studied *Aureobasidium pullulans*, a group of yeast and yeast like fungi, including *Rhodotorula aurantica* and *Kluyveromyces spp*. The present study shows that fungi have the ability to degrade synthetic polymer, especially polypropylene . Rawte et al. [12] studied the incidence of marine and mangrove bacteria accumulating polyhydroxy-alkanoates on the mid-west coast of India. The present study shows that aquatic microbes, especially fungi isolated from the east-coast, have the ability to show degradable activity on synthetic polymer polypropylene [12]. Microbial degradation of poly (caprolactone) (PCL)-poly vinyl butyral (PUB). Present studies show that Microbial degradation of synthetic polymer polypropylene (PP) is also possible by fungi isolated from the soil samples collected from different environments having different physio-chemical properties [13]. Biodegradation of low-density polyethylene (LDPE) by mangrove fungi from the red sea coast. He states that six isolates from Saudi Arabia were found to be able to grow in association with LDPE film under invitro conditions in the absence of dextrose or any other carbon source [8]. The present study shows that fungi isolated from the east coast Bay of Bengal and Indian ocean region have the ability to grow with the polypropylene (PP) film under in vivo conditions in room temperature.

The plastic degrading fungi *Trichoderma viridae* and *Aspergillus nomius* isolated from local landfill soil in Medan" [9]. This experiment states that the weight of LDPE used in the experiment was reduced by using the fungi *T. viridae and A. nomius* (5.13% and 6.63%), respectively, after 45 days of cultivation. Present study shows that synthetic plastic degradation by fungi has reduced its weight within 14 days of incubation. JMR da Luz et.al, [6] studied Plastic polymers degradation by fungi. Their experiment on fungi degradation of plastic by using *P.ostreatus* and sunlight to degrade plastic under abiotic and biotic combination has shown to degrade plastic due to the activities of lignocellulolytic enzymes that are produced during fungal growth on plastic sheets. The present study shows that fungi growth under biotic conditions can degrade an abiotic compound such as synthetic polymer polypropylene [15].Fungal degradation and valorisation of plastic waste". He states that the presence of depolymerase in certain microorganisms helps to break down the long polymer chain in the synthetic polymers. Present studies show that Fungi has the ability to depolymerase the long polymer chain of polypropylene [10] . Asiandu AP et al. [2] "Biodegradation of plastics waste using Fungi". She states that *Apergillus spp*., and *Penicillium Spp*. have the ability to degrade fungi. The present study shows that strains isolated from the east coast of India have the ability to degrade microplastic made out of synthetic polymer polypropylene and show degrading activities within a very short period of time.

CONCLUSION

Microplastic is a threat to our world especially marine ecosystem. Widespread of plastic floating over the debris have caused huge destruction in the marine ecosystem. Especially synthetic plastic which are non-biodegradable are main reason for the marine pollution. The current research demonstrates the isolation, characterization and myco-degradation analysis of degrading synthetic plastic polypropylene (PP) by the fungal strains do not require prior oxidations or other chemical treatments. It shows that fungi isolated from different environmental conditions has the ability to show degradable activity on tough synthetic thermal polymer such as polypropylene in a very short period of time. This shows that certain exoenzyme release from these fungi isolates from different environmental conditions can break the long polymer chain and depolymerase it in a very short period of time. Previous studies have shown this kind of activities only by using bacteria present studies have proven that aquatic fungi also have the ability to show degrading factors such as cracks, holes, scions, dents at a faster rate within a short period of time. Fungi was able to degrade the synthetic polymer such as polypropylene through chain reactions, via free radicals which degrade the polymer properties, with the polymeric chain and polymeric chain branch disruptions. Thus, the information procured acts as a shred of evidence for the degradation capability of isolated fungi on degrading synthetic polymer polypropylene (PP) which can be further enhanced in an industrial scale for degrading various plastic materials.

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COMPETING INTRESTS

"The author have declared that no competing interest exists".

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