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Enrichment and isolation of micro plastic degrading microorganisms from various natural sources

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ABSTRACT

Microplastics have pure Carbon backbone which makes them resistant towards degradation by conventional treatment systems. Microbes have shown the potency to degrade the microplastics. This study aims to isolate microplastic degrading microorganisms from various natural sources. Various soil samples and water samples were collected to isolate the microplastic degrading microorganisms. There are many different types of microplastics but polyethylene and polypropylene were targeted for the study. Mineral Salt Media with 0.1% polyethylene/polypropylene as a sole carbon source were used. 4 different isolates degrading microplastics have been isolated.

Key words: *Mircoplastics, Plastic degradation, Polyethylene, Polypropylene.*

Introduction

The extensive production and overuse of plastics have led to 'plastic pollution' which affects the environment by its ill effects on the wildlife, wildlife habitats and humans. The fragments of platics that are smaller than 5mm in any dimension are considered as microplastics. The formation of microplastics is by slow degradation process and mechanical wear and tear of plastics. The components of microplastics include polypropylene (PP) (15.5%), polyethylene (PE)(54.5-65%) and polyethylene terephthalate (PET)(2%). The microplastics are resistant to degradation and has gained attention due to harmful effects to living ecosystem.

Now-a-days plastics have replaced various components like wood, glass, metal, etc. as it is cost effective. Hence, its production has increased. But disposal of the plastics is the subject of concern. If the plastics are not disposed properly it can cause various environmental problems. There are various traditional methods for plastic disposal like incineration, landfilling, etc. The accumulation of plastics will not let the the air and water to go into the earth which will further cause soil infertility (Divyalakshmi and Subhashini, 2016). The other traditional methods have other various hazardous effects on the environment. Hence, biodegradation is the only safe option without any drawbacks.

Materials and Methods

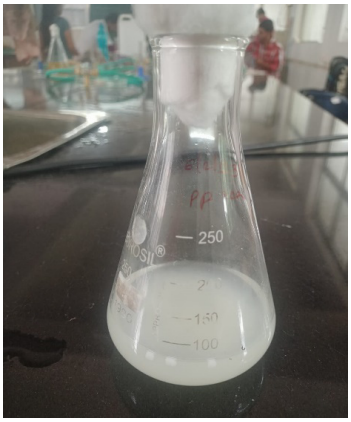
Collection of samples

Various soil and water samples were collected from different sites like seawater, water sample from dairy industry, soil sample from plastic dumping grounds, etc. Additionally, small pieces of polyethylene and polypropylene was buried 10-20 cm below in garden soil and was allowed to be there for several months and periodically soil from that site was sampled after 20 days, 40 days , 60 days and

after 80 days surrounding the polyethylene and polypropylene pieces.

Enrichment of polyethylene/polypropylene degrading microorganisms

The samples collected from various sources such as sea water, soil from plastic dumping grounds, water from dairy industry were subjected to enrichment of microplastic degrading microorganisms using mineral salt liquid culture medium with polyethylene/polypropylene as the only carbon source for enrichment. For enrichment of plastic degrading microorganisms from the samples of soil, water, etc samples were inoculated separately into different flasks containing liquid culture nutrient medium supplemented with polyethylene and polypropylene at the concentration of 0.1% W/V and were incubated at 30 °C for 2-4 weeks.



Photoplate 1. Enrichment of sample in MSM with microplastic as Carbon source:

Photoplate 2. Cut and sterilized Polyethylene plastic pieces

The samples from enrichment flasks were then kept ready for isolation of organisms using solid media.

Photoplate 3. Cut and sterilized polypropylene plastic pieces

Isolation of polyethylene/polypropylene degrading microorganisms

One loopful from enrichment culture flask was streaked on mineral salt agar medium supplemented with polyethylene/polypropylene and incubated at 30°C for 2-4 weeks. Subculturing was done of the colonies with different colony morphology onto Nutrient agar plates for purification of isolates.

Results and Discussion

Collection of sample

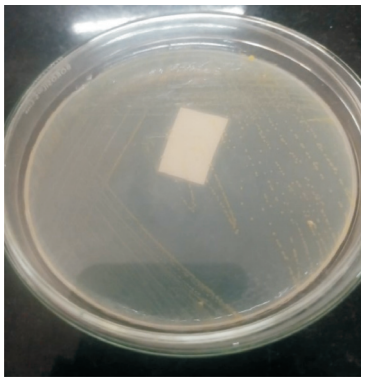
Sr. No.	Sample type	Source of sample
1	Soil	Plastic dumping grounds
2	Water	Sea water

All the samples were added to enrichment media and after 30 days of incubation turbidity was observed

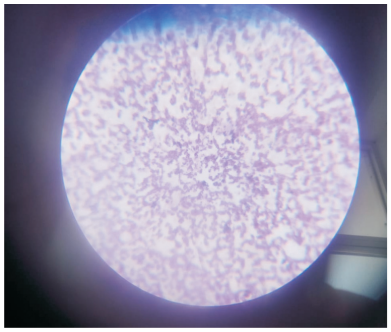
Isolation of microplastic degrading microorganisms

After the enrichment of samples, a total of 4 different isolates were obtained among which 2 were bacteria and 2 were fungi. Isolation of microorganisms was done on MSM and nutrient agar.

As per the Table 1, A total of 2 bacterial isolates were obtained. The bacterial isolates were designated as isolate 1 and 2. The colonies of isolate 1 were yellow coloured, circular shaped and convex elevation. The Gram nature of isolate 1 was gram positive cocci. Isolate no. 2 was circular, convex, opaque, Mucoid and off-white in color. The Gram nature of isolate 2 was Gram positive cocci



Photoplate 4. Culture of Isolate 1

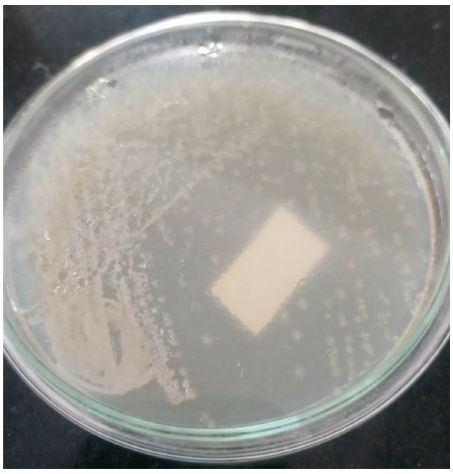


Photoplate 5. Microscopic examination of isolate 1

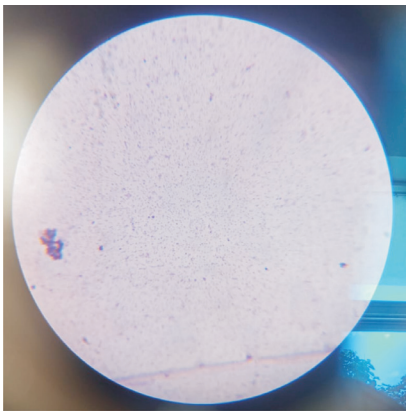
(Photoplate 4, 5, 6, 7).

Table 2) Morphological and colony characteristics of fungal isolates

The first fungal isolate was of *Aspergillus* spp which was septate and had presence of foot cell and conidiospore producing conidia. The second isolate was of *Penicilium* spp which was septate and had conidiospore producing conidia and showed broom like appearance of conidiophores (Photoplates 8 and 9).



Photoplate 6. Culture of isolate 2



Photoplate 7. Microscopic examination of isolate 2

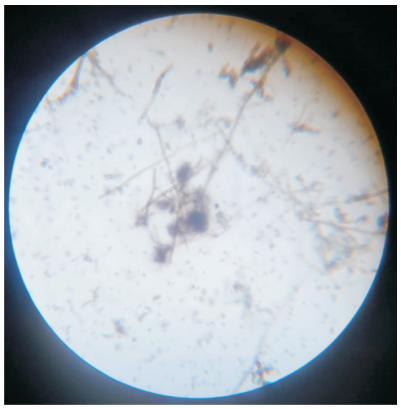
Table 1. Morphological and colony characteristics of the bacterial isolates

Isolate No.	Size	Shape	Margin	Elevation	Color	Opacity	Consistency	Gram nature
1	Pinpointed	Circular	Entire	Convex	Yellow	Opaque	Brittle	Gram +ve cocci
2	2mm	circular	irregular	convex	Off white	Opaque	Mucoid	Gram +ve cocci

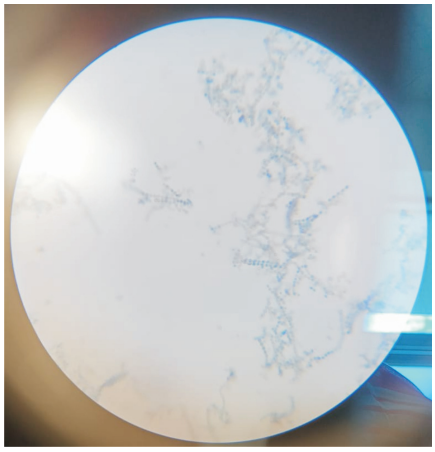
Table 2. The fungal isolates from enrichment:

Sr. No.	Colonial characteristics	Hyphae	Rhizoid	Sporangia/ Conidia	Sporangiospore/ Conidiospore	Fungi spp
1	Moderately rapid growing colonies that appeared black	Septate	Present	Conidia	Conidiospore	*Aspergillus* spp
2	Moderately fast growing colonies that appeared green	Septate	Absent	Conidia	Conidiospore (Broom shaped)	*Penicilium* spp

(Morphological and colony characteristics of fungal isolates)



Photoplate 8. Microscopic examination of fungal isolate 1.



Photoplate 9. Microscopic examination of fungal isolate 2.

Conclusion

The results obtained from the research confirmed that the four microorganisms have the capacity to degrade plastics. 2 bacterial isolates (Gram +ve cocci) and 2 fungal isolates (*Aspergillus* and *Peniclium* spp) were promising isolates.

Further work of this research includes,

1) Screening of the microorganisms degrading microplastic.
2) Determination of efficacy of the isolates for microplastic removal from wastewater treatment plant effluents.
3) Optimisation of the process of microplastic removal from wastewater treatment plant effluentusing the consortia.

Conflict of interest

No conflict of interest is there amongst the authors.

Acknowledgement

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