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Potential of Soil Fungi (*Penicillium* sp.,) to Form Biofilm on Polyethylene Terephthalate Surface

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Licentious and repeatedly deliberate release of plastics (Polyethylene terephthalate) is responsible for growing environmental pollution. In this study, *Penicillium sp*., has been isolated from PET (Polyethylene terephthalate) waste and its potential to degrade PET under laboratory conditions have been evaluated through electron microscopic studies. The observation of the present investigation reveal that *Penicillium sp*. were able to successfully colonies PET flakes surface and induce micromorphological changes like formation of multi-layered dense network of hyphae, ducking, surface corrosion and crystals which was evident in the SEM images.

Key words: Nitric Acid, PET, Biodegradation, *Penicillium* sp.

Polyethylene Terephthalate (PET) is a semi crystalline thermoplastic polymer, which is used in the preparation of a variety of products differing widely in their physical characteristics and hence, the end uses The varieties of prominence are fibres and filaments, sheets and soft drink bottles Gargiulo *et al*., (1997). PET bottles brings a lot of convenient to peoples life as a result of the diversity of its applications in a high volume of consumer products, large amount of PET waste is also generated, which includes polymer manufacturing waste as well as the products after the end of their useful life. With the increasing pressure of keeping the environment clean, recycling the PET waste in an ecofriendly manner is the only solution. (Ishihara *et al*.,1999 ; Sato *et al*., 1995).

The soil conditions determine the number of biodegrading microbial species and thus their population strongly affects overall biodegradation (Kimura *et al*., 1994). It is known that fungi and bacteria, which are the major component of the biosphere are responsible for breakdown of organic compounds and circulation of elements in the environment (Lucas *et al*.,2008). These microorganisms with the ability to adapt to diverse and changeable environmental conditions are able to degrade even totally new synthetic compounds. Therefore, attempts to raise the susceptibility of plastic to degradation are mainly aimed at the action of microorganisms. Fungi attack plastic polymers, *Cladosporium resinae* was found to degrade polyurethane (Gautam *et al*., 2006; Jecu *et al*., 2010). A few years ago the thermophilic actinomycete *Thermobifida fusca* was isolated and the polyester degrading enzyme was characterized to be a hydrolase combining the properties of lipase and esterase (Gouda *et al*., 2002; Kleeberg *et al*., 2005)**.** These provide an additional carbon source for the fungi and may also provide access points for the fungi to invade the synthetic polymer (Jecu *et al*., 2010)**.** Also works were focused on polymer degradation by fungi, *Fusarium*, *Aspergillus*, *Phanerochaete chrysoporium* and it is of interest to use the potential of fungal strains,

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because they are versatile organisms able to grow and degrade a variety of compounds, organic contaminants and polymeric materials (Chiellini *et al*., 2003). The present study aims to visualise electron microscopically the event of changes that has occurred during the PET degradation process by *Penicillium sp.*

MATERIALS AND METHODS

Sample collection for isolation of fungi

PET water bottles with soil samples (PET water bottles waste rich in soil) were collected from garbage in Tiruchiarappalli, Tamil Nadu. They were cut into small sizes 1cm × 1cm. washed with distilled water and inoculated in minimal salt medium at 120 rpm for 24 hour in orbital shaker at room temperature to isolate fungi associated with PET waste by pure plate method using, Rose Bengal Chlormophenicol Agar. Further, plates were incubated at 28^oC for 7 days and developed colonies were isolated and sub-cultured to get pure colonies and stored in refrigerator for further studies (Nigam,1965; Warcup,1950).

Biodegradation in Nutrient Broth medium

Drinking water bottle (PET) was collected from shop and was cut into small flakes about 0.5×0.5 mm size and were washed with distilled water. Further, they were treated with chemical (boiled with 100^0C of Nitric acid for 15 minutes). Finally, PET strips were washed thoroughly with 70% ethanol and finally washed with distilled water thereafter kept in oven at 50^0C for one hour. Further, they were inoculated into nutrient broth medium along with *Penicillium sp.*, for a period of one month.

Scanning Electron Microscopy (SEM)

The scanning electron microscopy analysis of surface of PET film was carried out using Scanning electron microscope (TESLA B340). The surface of the treated PET samples were coated with conductive heavy metals such as gold/ palladium.

RESULTS AND DISCUSSION

To check the existence of morphological changes as a result of *Penicillium sp.* degradation, the PET samples were analysed by SEM. A comparative assemblage of scanning electron micrographs of the untreated and HNO_3 treated samples of PET flakes inoculated with *Penicillium sp. is* depicted in fig-1 to fig-3. From the fig-1, it is evident that the untreated PET flake sample surface was smooth without any cracks or fracture and were free from defects. Structural changes such as cracks and ducking were observed in HNO_3 treated PET flake (Fig-2). However, exposure to *Penicillium sp*., resulted in a multi-layered dense network of hyphae, sporangia and spores (Fig-3,3a). The observations of the present study reveals that *Pencillium sp*., could be used to degrade PET flakes.

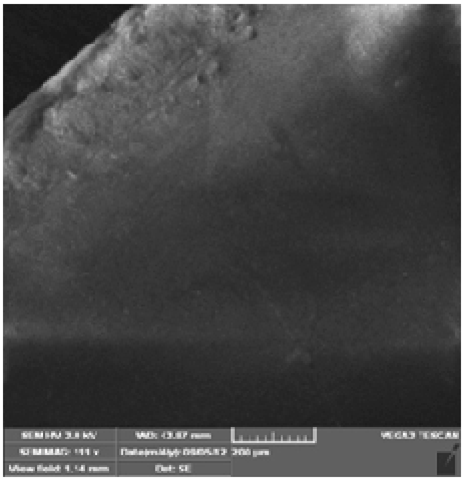

Fig. 1. SEM of untreated PET flake sample

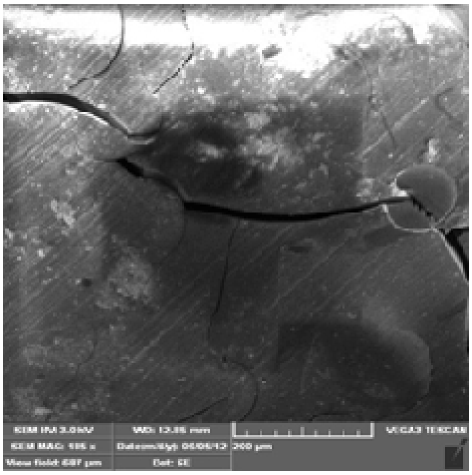

Fig. 2. SEM of Nitric acid treated PET flake sample

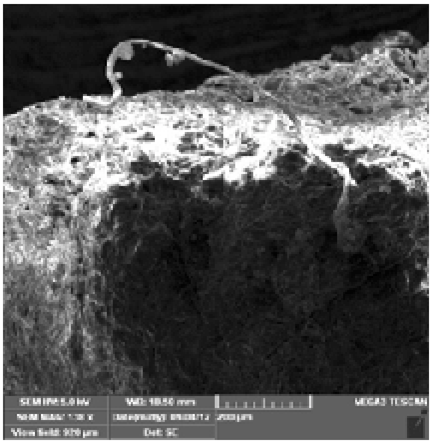

Fig. 3. SEM of *Penicillum sp.*, inoculated PET flake sample

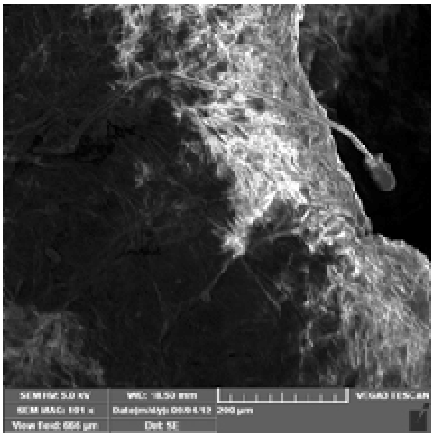

Fig. 3a. SEM of *Penicillum sp.*, inoculated PET flake sample

This finding is in good accord with reports of Eya *et al.*, (1999) who have stated that plastic degradation might result from attack by the soil micro flora in soil Kimura *et al* .,(1994) have emphasized that the degradation of plastic was mainly caused by bacteria and fungi and different soil conditions affected the rate of degradation of plastics. Our finding agrees with that Pramila and Vijya Ramesh (2011) who have observed colonisation of fungi *Asperigillus flavus* and *Mucor circinelloides* on the surface of LDPE (Low density polyethylene), which has caused some physical changes. According to lowe (1992), the biodegradation of poly(vinylalchol) composite films was tested using *Aspergilllus niger* . Fungi are widely used in biodegradation studies due to their robust nature and for their great source of diverse enzymes. The species element of a fungi growth are observed in the most relevant SEM micrograph of *Apserpillus niger* culture on PVA composite. SEM is a significant and reliable tool to measure the morphological changes of degraded polymer (Labuzek *et al.*,(2004)**.** Imam *et al*.,(1999) have observed that significant biodegradation occurred only after colonization of plastic, a parameter that was dependent on the resident microbial population.

CONCLUSION

The observations of the present investigation reveal that PET waste dumped in soil is exposed to a variety of organism, especially fungi. *Penicillium sp.,* isolated from PET waste were reinoculated with nitric acid treated PET under laboratory conditions. The growth of *Penicillium sp.,* on PET was evinced in the SEM images. Thus ,these findings permits us to conclude that fungi are able to colonise and form biofilms on PET surface which indicates that fungi are able to utilize PET as a carbon source for their growth. However, the involvement of fungi in degrading PET has to be confirmed by further studies.

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