SCREENING OF MICROBIAL CONSORTIUM FROM BIOFILMS ON DEGRADING PLASTICS FOR THEIR PLASTIC DEGRADING EFFICIENCY

Project Reference No.: 47S_MSC_0201

College ***:*** Karnatak University, Dharwad
Branch ***:*** *Microbiology*
Guide(S) ***:*** *Prof. V. Shyam Kumar*
Dr. Sheela Khanapure
Student(S) ***:*** *Mr. Jatteppa Alal*
Mr. Nagaraj Basavaraj Marihal
Mr. Neelakanth Mantur
Mr. Rakesh Abbigeri

Keywords

Biofilms, LDPE, Microbial consortium

Introduction

Plastic has become a very fundamental part of our society despite being a severe threat to the environment, (Danso *et al.,* 2019). Due to its lightness, durability, inertness and cheap production costs, etc., It is widely used in various fields ranging from industries, and agriculture to our day-to-day life, (Orr *et al.,* 2004; Sudhakar et al., 2008; Yuan *et al.,* 2020). Worldwide the overall production of plastics usage was reported at around 367 megatons in 2020 and year by year the number is increasing exponentially due to its efficient and versatile use (Plastics Europe, 2021). Plastic endangers aquatic and terrestrial wildlife (Gall and Thompson, 2105: Blettler and Mitchell, 2021) and harms soil fauna (Huerta Lwanga *etal,* 2016).

Microbes are found to colonize on the surface of the high molecular weight plastic polymers, such as LDPE causing biofilm formation which is one of the important steps during microbial degradation of these plastics (Sivan, 2011; Haider *et al.,* 2016). Hence, the current study is focused on screening the microbial consortium from biofilms of degrading plastics from water bodies to check their efficacy of plastic degradation.

Objectives

- To isolate microbes (Bacteria, Fungi) from biofilms formed on degrading plastic infreshwater bodies
- Biochemical &Molecular Characterization of Potential Microbes
- Evaluation & characterization of macromolecules from Microbes for Plastic degradation efficiency

Methodology:

- Study location and collection of samples: The sampling spot is located in Dharwad District. It is highly contaminated with polyethylene waste due to the huge population and their needs. Biofilms formed on plastic in water bodies were collected from the lakes (Nuggikeri lake, Kelageri lake etc) in and around the Dharwad area for microbial analysis and stored at 4 ˚C until use.
- Isolation and maintenance of pure culture: Different media were used to isolate the plastic degrading bacteria (Minimal media, Specific media).

- After successful growth of the microorganisms, each single colony will be identified (based on colony morphology colour) and re-streaked as primary inoculants on the surface of the NA media (bacteria), SDA(Fungi). The plates will be then incubated at27 ± 2 ^{o}c for 1 to 2 days and stored at low temperature (4 ± I ^{o}c) for bacteria and 37 ±2 ^{o}c for 3 to 5 days for fungi for further use.
- Low density polyethylene Fresh low-density polythene carry bags obtained from Dharwad market were used for this study. LDPE bags were cut into (3x3 cm) pieces and then washed with 70% ethanol for 30 min, then followed by distilled water, and air dried for 15 minutes in the laminar air flow chamber and then used for future studies.
- Screening of Low-density polyethylene (LDPE) degrading microbes through clear- zone formation: The agar/SDA plate is emulsified with LDPE, a pure bacteria/fungi culture will be spread over it, and then, incubate the plate at 37 ^{o}C for 1 to 2 days for bacteria and 30 ^{o}C for 3 to 7 days for fungi, Plate will be observed for clear zone formation indicating plastic degradation the zone of clearance will be measured with scale. Based on diameter of the zone the microbes will be screened for further degradation studies.
- Fourier transform infrared spectroscopy analysis: FTIR analysis will be performed to detect the formation of new functional groups or changes in the amount of existing functional group. After the incubation period, the sample will be collected and washed with water and followed by ethanol to remove debris and again washed with distilled water to remove excess precipitation and then allowed it to dry. The surface changes made on LDPE pieces will be analysed through FTIR studies.
- Scanning Electron Microscopy analysis: The treated samples after a period of incubation will be washed with 2 % (v/v) aqueous SDS and distilled water for few minutes and flushed with 70 % ethanol to remove the cells. After that the sample will be pasted onto the SEM analysis stub using a carbon tube and the sample will be analysed under high-resolution scanning electron microscope.

Result and Conclusion

Since the project is under progress, we are expecting microbial consortium of biofilm collected on the LDPE's could degrade a certain amount of plastic (Fig 1 and 2). The degradation of plastics will be confirmed by subjecting them to FTIR and SEM analysis.

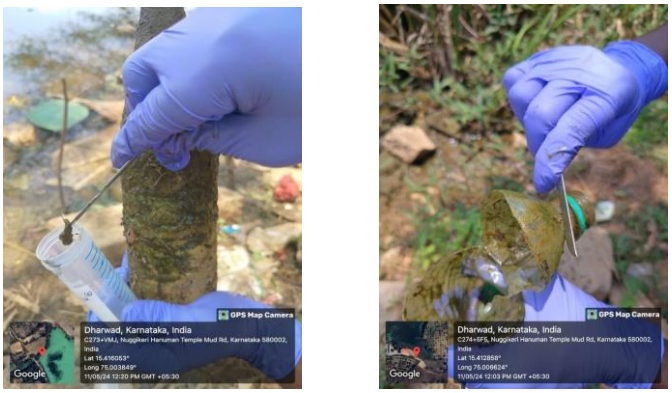

Figure 1.1-Sample collection from Nuggikeri lake (Biofilm scraped from the plastic bottles and plastic residues found in the water bodies)

Figure.1.2- Samples collected from water bodies inoculated into the enrichment media (Minimal Salt Media).

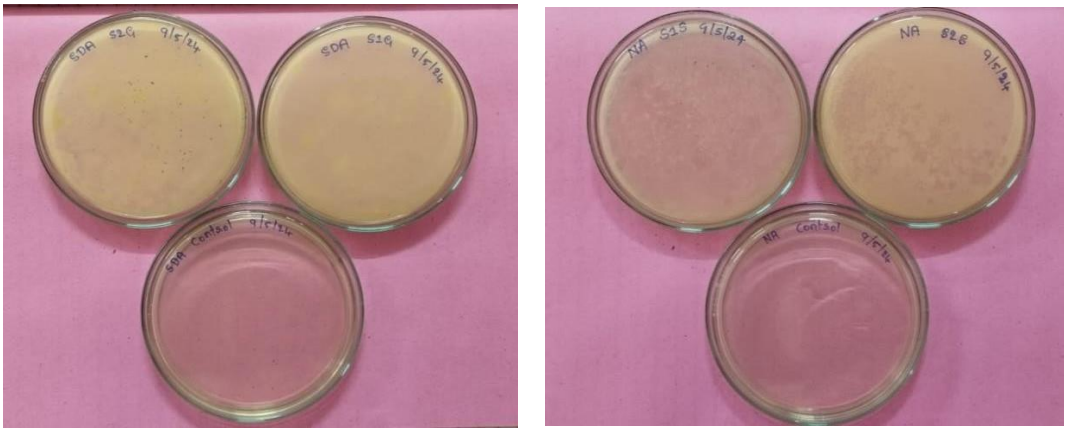

Figure 1.3- Sabouraud's Dextrose Agar media and Nutrient Agar media plates inoculated with the inoculum obtained from the enrichment culture technique using Minimal Salt Media (MSM). Post incubation indicating the presence of microbes in MSM media

Innovation in the project

This project provides innovative approach of the biodegradation of plastics by microbial consortium found upon the biofilm formed on the plastics in water bodies. This eco-friendly and cost-effective idea is not only limited to lab-scale but can also be scalable to an industry level.