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Isolation And Molecular Identification Of Polyethylene Degrading Bacteria From Soil And Degradation Detection By Ftir Analysis - Haghi Morteza - EDGE Food Control and Research Laboratory

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Abstract

Today, the increase in plastic waste accumulation is an inescapable consequence of environmental pollution, the disposal of these wastes has caused a significant problem. variable methods have been utilized, however, biodegradation is the most environmentally friendly and low-cost method. Accordingly, the present study aimed to isolate the bacteria capable of biodegradation of plastics. In doing so, we applied the liquid carbon-free basal medium (LCFBM) prepared with deionized water for the isolation of bacterial species obtained from soil samples taken from the Izmir Menemen region. Isolates forming biofilms on plastic were selected and named (PLB3, PLF1, PLB1B) and subjected to a degradation test. FTIR analysis, 16s rDNA amplification, sequencing, identification of isolates were performed. Finally. At the end of the process, a mass loss of 16.6 % in PLB3 isolate and 25 % in PLF1 isolate was observed, while no mass loss was detected in PLB1B isolate. Only PLF1 and PLB1B created transparent zones on plastic texture. considering the FTIR result, PLB3 changed plastic structure by 13.6% and PLF1 by 17%, while PLB1B did not change the plastic texture. According to the 16s rDNA sequence analysis FLP1, PLB1B and PLB3 isolates were identified as Streptomyces albogriseolus, Enterobacter cloacae and Klebsiella pneumoniae respectively. The level of biodegradation of LDPE sheets with bacterial and fungal inoculums from different sampling points of Dandora dumpsite was evaluated under laboratory conditions. Incubation of the LDPE sheets was done for sixteen weeks at 37°C and 28°C for bacteria and fungi respectively in a shaker incubator. Isolation of effective candidates for biodegradation was done based on the recorded biodegradation outcomes. The extent of biodegradation on the polyethylene sheets was assessed by various techniques including weight loss analysis, Fourier Transform Infrared Spectroscopy (FTIR) and GC-MS. Fourier Transform Infra-Red spectroscopy (FTIR) analysis revealed the appearance of new functional groups attributed to hydrocarbon degradation after incubation with the bacteria and fungi. Analysis of the 16S rDNA and 18S rDNA sequences for bacteria and fungi respectively showed that bacteria belonging to genera Pseudomonas, Bacillus, Brevibacillus, Cellulosimicrobium, Lysinibacillus and fungi of genus Aspergillus were implicated as polyethylene degraders. An overall analysis confirmed that fungi are generally better degraders of polyethylene than bacteria. The highest fungal degradation activity was a mean weight reduction of 36.4±5.53% attributed to Aspergillus oryzae strain A5, 1 (MG779508). The highest degradation activity for bacteria was a mean of 35.72± 4.01% and 20.28± 2.30% attributed to Bacillus cereus strain A5,a (MG645264) and Brevibacillus borstelensis strain B2,2 (MG645267) respectively. Genus Aspergillus, Bacillus and Brevibacillus were confirmed to be good candidates for Low Density Poly Ethene bio-degradation. This was further confirmed by the appearance of the aldehyde, ether and carboxyl functional groups after FTIR analysis of the polythene sheets and the appearance of a ketone which is also an intermediary product in the culture media. To improve this degrading capacity through assessment of optimum conditions for microbial activity and enzyme production will enable these findings to be applied commercially and on a larger scale.

Biodegradation is the decomposition of substances through microbial activity and is a complex process which involves the following steps: biodeterioration,

depolymerization, assimilation, and mineralization. Bacteria and fungi of various genera have been implicated previously in the biodegradation of polyethene albeit the low rates. Acinetobacter sp. was found capable of utilizing n-alkanes of chain length C10–C40 as a sole source of carbon as reported. Bacterial genera, namely, Pseudomonas, Acinetobacter, Brevibacillus, Rhodococcus, and Micrococcus respectively, isolated from different sources proved to be the potential organisms for polyethene degradation. Fungal genera, Gliocladium, Cunninghamella, Penicillium, Aspergillus, Fusarium, Mucor, and Mortierella, from soil were proven to have the potential to degrade polyethene after analysis of degradation through various methods.

Plastic biodegradation as a result of the activity of certain enzymes causes cleavage of the polymer chains into monomers and oligomers. Enzymatically broken down plastic is further absorbed by the microbial cell to be metabolized. Aerobic breakdown produces carbon dioxide and water. The involvement of enzymes in microbial biodegradation of polyethene has been investigated, and enzymes such as laccases and esterases have been confirmed to play a role in this process either directly or indirectly. The production of enzyme laccase in the presence of polyethene as the sole carbon source is a clear indication that laccase has a role in breaking down some of the intermediary products produced during this process. In this study, molecular characterization of bacteria and fungi that had been confirmed to degrade polyethene was done as well as assessment of optimum pH, temperature, and sodium chloride concentration at which they can thrive.