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Isolation and Screening of Plastic Degrading Bacteria from Polythene Dumped Garbage Soil

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Abstract: ***Plastics are the most commonly used polymers for routine applications. Plastic wastes accumulating the environment are posing an ever increasing ecological threat. Low Density Polyethylene (LDPE) is a major cause of persistence and long term environmental pollution. The most eco- friendly approach to resolve this ever growing and persistent issue is the microbial degradation route. The main objective of the present study is to isolate and screen for bacteria having the capability to degrade low density polyethylene (LDPE). Bacteria were isolate from various garbage dumped soil. The isolates are grown in MSA medium contain plastic strips. The clear zone method to detect the PEG utilization. From these strains inoculated with Bushnell Haas Broth with pre-weighed LDPE plastic strip.SEM analysis and FTIR spectroscopy. Molecular characterization, BLAST and Phylogenetic tree. The isolate was found to be Bacillus sp. We can conclude that Bacillus sp. may act as solution for the problem caused by polythene in nature. Hence from this study it can be speculated that microbes has enough potential to degrade plastic with due course of time.***

Keywords: Plastics, LDPE, FTIR, SEM, BLAST, Bacillus sp

I. INTRODUCTION

The word plastic comes from the Greek word "plastikos", which means 'able to be molded in to varied shapes'. Plastics are polymers that consist of monomers linked together by chemical bonds[11]. Plastics are advantageous as they are strong, light-weight, and durable. However, they are disadvantageous as they are resistant to biodegradation, leading to pollution, harmful to the natural environment [1]. The microbial degradation of plastic is carried out by enzymatic activities which lead to the breakdown of polymer into monomers and oligomers followed by metabolism by microbial cells [9]. Microorganisms such as bacteria, fungi and actinomycetes degrade both natural and synthetic plastics [2]. The richness of microbes able to degrade polythene is so far limited to 17 genera of bacteria and 9 genera of fungi [3]. Microbial degradation of plastics is caused by oxidation or hydrolysis using microbial enzymes that leads to chain cleavage of the large compound polymer into small molecular monomer by the metabolic process [6].The aim of the present study was to isolate microorganisms from varied natural sources and screen them for potential polyethylene degrading capability. The objective of the work carried out was to identify and characterize the high potential LDPE degraders. The isolation was carried out through serial dilution method of samples from garbage dumped soils from various locations. The screening of potential LDPE degraders was carried out using a sequential screening procedure using Clear Zone method followed by In-Vitro Biodegradation assay and determination of dry weight of residual polyethylene.Based on 16S rRNA analyses the isolated strain was identified as Bascillussp. LDPE degradation by Bascillussp was monitored for 45 days of incubation in aqueous medium. The degradation was confirmed by changes in polyethylene weight, and morphological changes by SEM and FTIR analysis.

II. MATERIALS AND METHOD

A. Sample Collection

Soil samples were collected from different garbage dumped areas of Kolenchery.

B. Serial Dilution Method

1.0 gram of soil sample was transferred into a conical flask having 99ml of normal saline. The mixture was shaken and serially diluted.

C. Petriplate Method

From the dilution plates isolated bacterial colonies are pick and streaked on minimal salt agar. Polythene strips of 2x2 cm were cut and placed on the minimal salt agar plates. After the incubation the growth of microorganism were seen on the polythene strips[8].

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D. Screening of Polythene Degrading Microorganism

In this method 1% concentrations of PEG were used in minimal salt agar media.0.1ml culture organism were spreaded on the petriplates.

E. Clear Zone Method

Isolates were screened for LDPE degrading efficiency by zone of clearance method [5]. After the completion of incubation period, agar plates were flooded with 0.1% (w/v) Coomassie blue R-250 solution in 40% (v/v) methanol and 10% (v/v) acetic acid for 20 minutes. The solution of Coomassie blue was then poured off, and the plates were flooded with 40% (v/v) methanol and 10% (v/v) acetic acid for 20 minutes. The organisms producing zone of clearance in a blue background were selected as the utilizer of polyethylene [7].

F.In-Vitro Biodegradation Assay

Bacterial isolates were grown overnight.1ml culture was inoculated in 250ml Erlenmeyer flask containing 100ml of Bushnell Haas broth (autoclaved) and polyethylene films. Prior to transfer to liquid culture media, polyethylene films were cut in to pieces (3x3cm), disinfected with Tween 20, distilled water and 70% ethanol for 30min, air dried for 15min in laminar air flow chamber. Weighed the polyethylene strips and added to the flask. As for control, uninoculated minimal broth supplemented with LDPE films were maintained under similar conditions. And the flasks are placed in a rotary shaker [10].Different flasks will be kept in a shaker for 15, 30 and 45 days respectively.

G. Determination of Dry Weight of Residual Polyethylene

To facilitate accurate measurement of residual polyethylene weight, bacterial biofilms were washed off the polyethylene surface with 2% (v/v) Sodium Dodecyl Sulfate (SDS) overnight, followed by rinsing with distilled water [5].

$$\text{Weight loss \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

H. Characterization and Identification of Microorganism

Five isolates selected from the clear zone method. The isolates were characterized by various biochemical tests and gram staining method. From the five samples the best plastic degrading one strain is taken for further analysis.FTIR spectroscopy performed to chek the biodegradation of polyethylene. FTIR analysis was carried out using infrared spectral data collected on Thermo Avatar 370 FTIR spectrometer. The data was recorded in the spectral range of 4000–400 cm^{-1}at 4 cm^{-1} resolution with an inter ferogram of 32 scans.SEM analysis was carried out for obtaining more structural information about polyethylene strip. The sample is smeared on a small piece of adhesive carbon tape which is fixed on a brass stub. The sample, then subjected to gold coating using sputtering unit (model: JFC1600)for 10 sec at 10mA of current. The gold coated sample placed in chamber of SEM (Jeol, JSM 6390LA) and secondary electron/Back Scattered electron images are recorded. Molecular characterization and Phylogenetic tree construction using MEGA 6 software.

III. RESULTS AND DISCUSSION

A. Clear Zone Method

In this procedure zone of clearance method was observed by staining with Coomassiee blue where the 1.0 concentrations of PEG was used in minimal media containing salts of ammonium and potassium. Out of 15 bacteria, 5 showed the positive results. Coomassie brilliant R250 is used for staining after destaining clear zones are observed. These clear zones are formed by the utilization of PEG as carbon source. If PEG is not utilized by organisms it seen in blue colour.

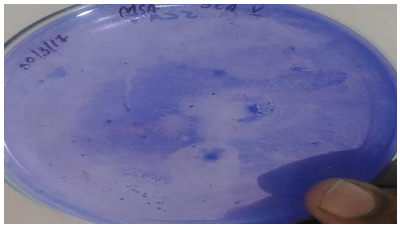

Figure1 Clear zone method

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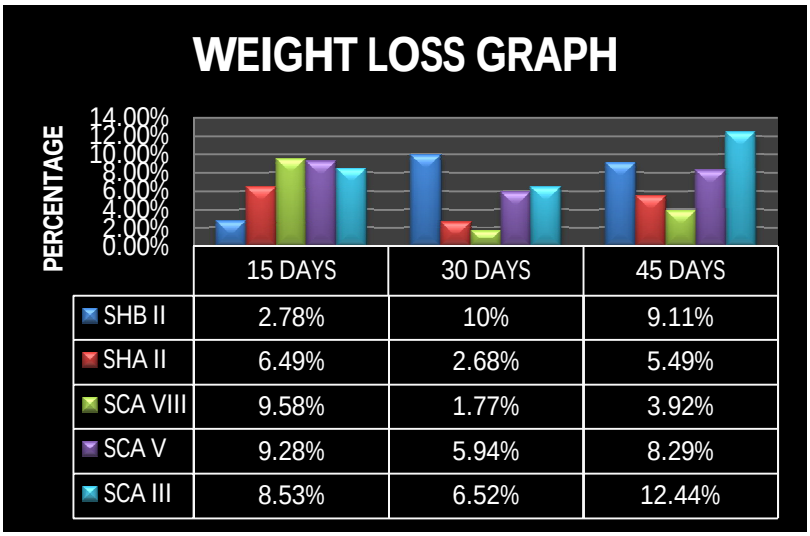
B. Weight Loss Graph



	15 DAYS	30 DAYS	45 DAYS
SHB II	2.78%	10%	9.11%
SHA II	6.49%	2.68%	5.49%
SCA VIII	9.58%	1.77%	3.92%
SCA V	9.28%	5.94%	8.29%
SCA III	8.53%	6.52%	12.44%

Figure2 Graph showing the weight loss of plastic strips

C. Biochemical Results

Five samples are taken for biochemical tests (SHB II,SHA II,SCAVIII, SCA V, SCA III). Indole test all samples shows negative results. MR test SHB II and SCA VIII gives positive results and SHA II, SCA III, SCA V shows negative results. In VP test all samples gives negatives results. Citrate utilization test SCA VIII is positive all others are negatives. Catalase test positive SCA VIII and negatives are SHB II, SHA II,SCA V, and SCA III. In Oxidase test all strains are positives. Urease test SCA V is negative and other four samples shows positive results. In sugar fermentation test- Sucrose test SCA VIII is positive and others are negative. Lactose test all samples are showing negative results. Dextrose test SCA VIII is positive and all others are negative.

D. FTIR Analysis

The structural analysis is the important parameter to identify the structural changes which appear during degradation responsible for weight loss. FTIR is sensitive to local molecular environment and as a consequence has been widely applied to investigate the interactions between the macromolecules during LDPE degradation. FTIR analysis of the degraded LDPE films gives a close view of N–H stretching of aldehyde group at 3606.49cm^{-1}. C=O stretching of aldehyde group at 2847.45 cm^{-1}, C–O stretching of ether group at 1464.92 cm^{-1}and twisting deformation at peak 723.99 cm^{-1}.The result is qualitative and confirmed that the vibrations positions belongs to LDPE which further confirmed by the reviewed literature[4].

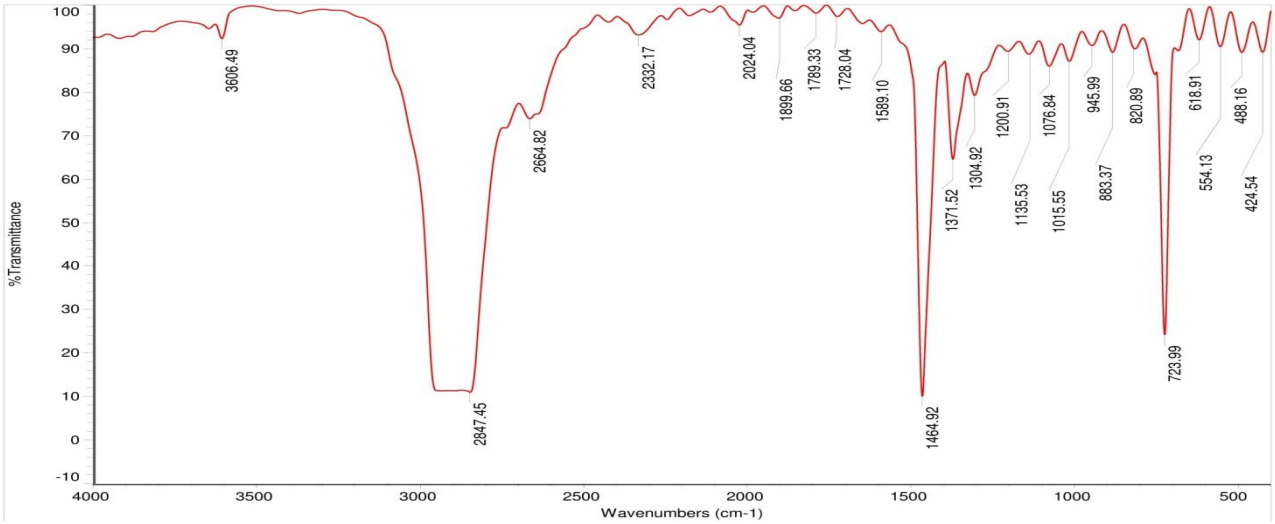


Figure3 FTIR analysis of LDPE strip- Sample (SCAIII)

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E. SEM Micrograph

SEM micrograph of the polyethylene strip incubated after 45 days with bacterial isolate SCA III was shown in figure4. SEM analysis is used to confirm that the surface of LDPE becomes physically weak after biological treatment. LDPE films exposed to bacterial isolate SCA III showed surface erosion, cracks and folding (Fig. 4). This may be due to the extracellular metabolites and enzymes released by the bacteria in response to stress. Surface bio-erosion is the primary cause of mass loss from surface.

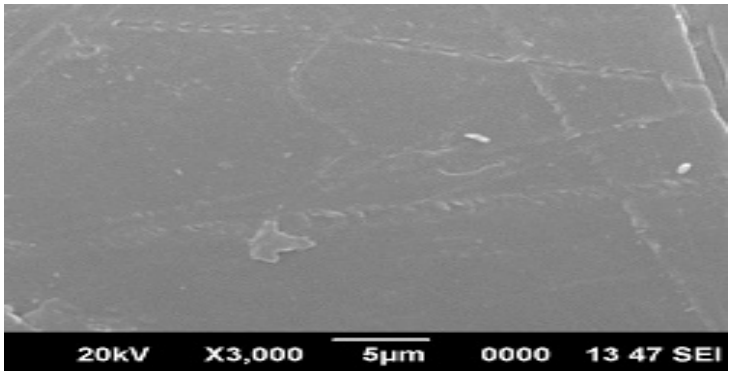


Fig 4.Sample (SCA III)

F. PCR amplification of the 16S rRNA and sequence determination

For amplification of 16S rRNA, universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3') were used. By using these primers a partial sequence of 16S rRNA gene was amplified. Genomic DNA was used as template for PCR amplification of 16S rRNA. A band of approx.1500 base pairs (bp) of the amplicons was observed in agarose gel.

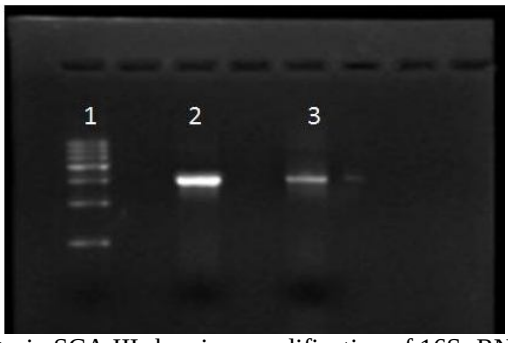
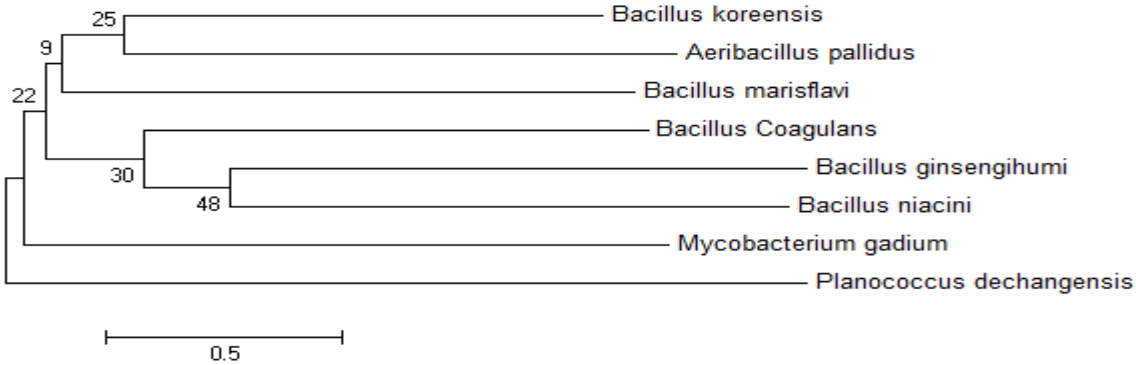


Figure: Gel of strain SCA III showing amplification of 16S rRNAamplicon.

Lane 1 showing 1 Kb DNA ladder
Lane 2 & 3 indicated band of amplicon with expected size of 1,500 bp



Phylogram of *Bacillus sp.* isolate based on 16S rRNA gene analysis and constructed by MEGA version 6.0.with respect to the closely related sequence available in Gene Bank

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IV. CONCLUSION

In recent years, public has shown interest and concern over environmental issues.The problem of plastic pollution is now really a mess of man kind.There is no part of the world untouched from impacts.In the presnt era of globalization some stress must be given to plan safe disposal of products before making it commercial.In the present study, FTIR spectroscopy and SEM micrograph shows the degradation of the LDPE strip. The sequence of PCR amplicon of best plastic degrading bacteria was compared using BLAST software which shown 99% identity with 32 *Bacillus* sp. and phylogenetic tree was also constructed using MEGA6 software. Isolation of the genes for the enzymes required for degradation of plastic could pave way for recombinant DNA technologies where recombinant strains which would degrade plastic in a shorter period can be developed.Thus the area of research for biodegradation of plastics by microbes should further be explored to gain new insight in the mechanism of action of microbial degradation of plastics.

V. ACKNOWLEDGMENT

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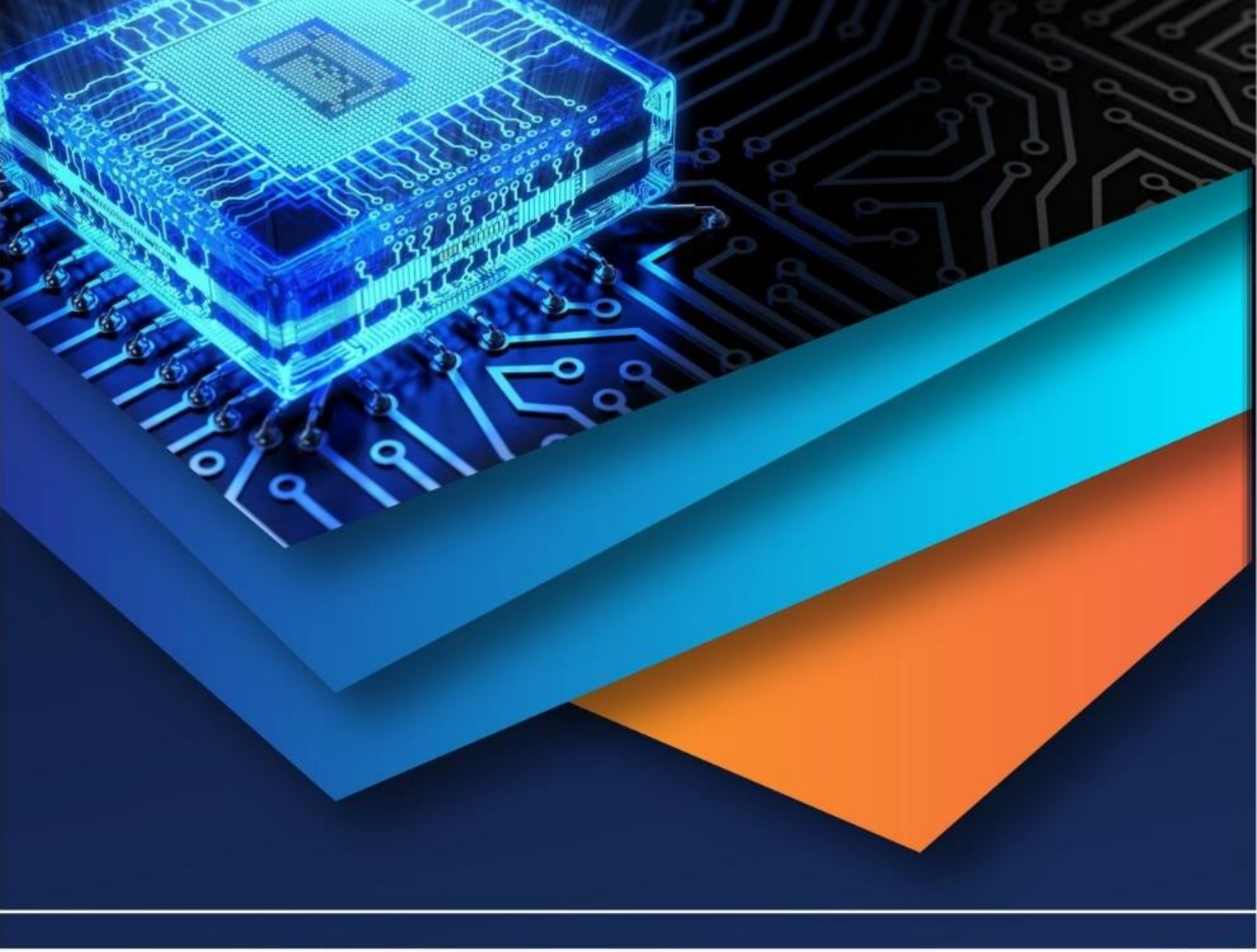

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