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Biodegradation of polythene by bacteria isolated from soil

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Abstract
Polythene is the most common plastic & it is the mixture of polymers & ethylene. Polythene play a vital role in packaging of goods, food material, medicine, garbage bags etc. and now a days its degradation is becoming a great threat against the cause of environmental pollution. There are various methods available for the degradation of polythene which are ecofriendly and more acceptable.
The present study deals with the isolation, identification, screening, and degradation of pretreated polythene by microorganism present from soil.
A total of 15 bacteria were recovered from different areas and further screening of polythene degrading microorganism was done by zone of clearance method and out of that 15 bacteria only 3 bacteria showed a positive result and identified to be *Staphylococcus sp., Pseudomonas sp., and Bacillus sp.* And according to that *Staphylococcus sp* showed 20% of degradation followed by *Pseudomonas sp* with 7.5% and *Bacillus sp* showed a maximum degradation i, e 42.5% in 40 days.
In this way we can conclude that the *Bacillus sp.* may act as a solution of problem caused by polythene in nature.

Keywords: Degradation, environmental pollution, and polythene

Introduction
Plastics are used in various purposes in our day to day life and due to that they cause the environmental pollution by accumulating in the environment. In most of the countries plastic pollution are caused due to in proper recycling and waste management system.
Biodegradation process play a vital role now a days to degrade and solve this problem.
Biodegradation is the process which includes microorganism like bacteria & fungi that can degrades the polythene. The microbial degradation of polythene is carried out by enzymatic activity which leads to breakdown of polymer into monomers and oligomers followed by metabolism by microbial cells. Microorganism utilize polythene film as a sole source of carbon resulting partial degradation of plastics.
Worldwide utilization of polythene is increasing at the rate of 12% per annum and due to that huge amount of polythene getting accumulated in the environment, so their disposal creates a big problem in term of ecology.
Many attempts were done to isolates polythene degrading bacteria from dumping yards. Near hospitals, local areas, and from petrol pumps.
Currently enzymatic degradation is most widely used for plastics waste treatment and biodegradation of microbial enzymes increases the rate of degradation of plastics without causing any harm to the environment.
It is very important to degrade the polythene from the atmosphere therefore an attempt has been made in this paper to isolate those microorganisms that can degrade the polythene.

Materials & Methods
Sample collection: Soil samples are collected by different areas of Dehradun.Polythene samples of different densities such as 10 microns & 40 microns were purchased.

Isolation of bacteria
Serial dilution method: 1.0 gram of soil sample transferred in conical flask having 99ml of distilled water. Mixture was shaken and serially diluted.

Petri plate method: Nutrient agar plates were prepared. Dilution were spread by spreading method on nutrient agar plates. Polythene strips of 3×3 were cut and placed on nutrient agar plates. After incubation the growth of microorganism were seen on polythene strips.

Screening of polythene degrading microorganism: This was carried out by zone of clearance methods where 0.5 concentration of PEG were used in minimal media containing salts of ammonium & potassium. Zone of clearance around the colonies were observed by staining with Coomassie blue. This indicates its capacity to utilize polythene as carbon source and degrades polythene.

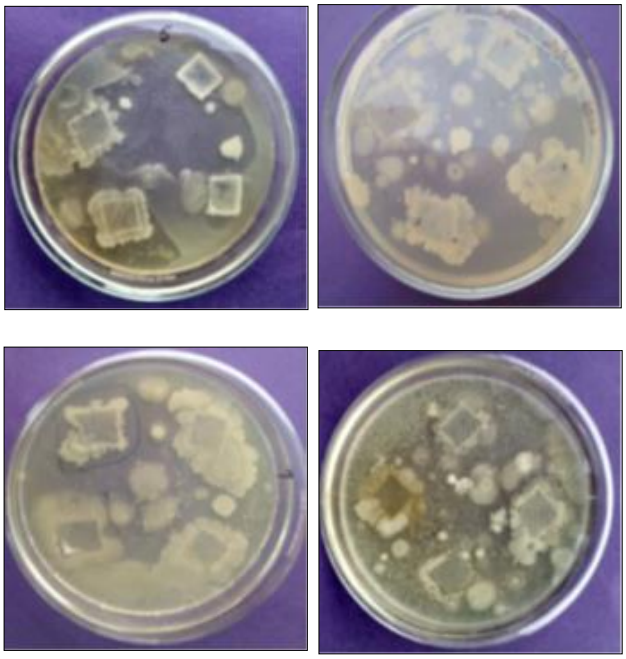
Characterization & identification of microorganism: After screening the isolates were characterized by various morphological and biochemical tests according to Bergey's Manual of Determinative bacteriology.

Pretreatment of polythene: Polythene bags were cut into small strips. Transferred into fresh solution having 70ml tween 80, 10ml bleach, 983ml of distilled water. Stir for 30 to 60 min. Strips were transferred into beaker with distilled water and stirred for 1 hour. Further aseptically placed in the ethanol solution 70% for 30 min. Polythene were transferred to a petri dish and used to disinfectant the polythene.

Degradation of pretreated polythene: Weighed strips of polythene were aseptically transferred to the conical flask containing 50ml of nutrient broth medium & inoculated

with bacteria (0.5ml). Different flasks will be kept in a shaker for 10,20,30,40 days respectively. After duration of shaking strips were collected Washed thoroughly using distilled water. Shade dried. Weighed for final weight. Percentage weight loss were calculated by formula.

Weight loss % = Initial weight – final weight / Initial weight



Results

A total of 15 bacteria were recovered from different areas. Areas selected were petrol pump, hospital, and local area.

Preliminary isolation of Bacteria

Screening of polythene degrading microorganism

1. **Primary screening:** Zone of clearance methods were observed by staining with Coomassie blue. Where 0.5 concentration of PEG is used in minimal medium containing salts of ammonium and potassium.

Table: Morphology and biochemical characterization of recovered isolates. Out of 15 bacteria 8 bacteria showed positive result

S. No.	Characteristics	P1A	P1B	P1C
01	Gram staining	Gram+ve Cocci	Gram-ve bacilli	Gram+ve bacilli
02	Shape	+	+	+
03	Nitrate reduction	-	+	-
04	Citrate utilization	+/-	-	+/-
05	Voges Proskauer	+	-	-
06	Methyl red	+	+	+
07	Catalase	-	-	+
08	Starch hydrolysis	Staphylococcus sp.	Pseudomonas sp	Bacillus sp
09	Identified isolates			

2. **Secondary screening:** Zone of clearance methods were observed by staining with Coomassie blue. Where 0.1 concentration of PEG is used in minimal medium containing salts of ammonium and potassium. Out of 8 bacteria 3 bacteria showed positive result.

Screening through Zone of clearance method

Characterization and identification of microorganism: Microorganism that showed positive results were identified as *Staphylococcus sp, Pseudomonas sp, Bacillus sp* and various results were seen by performing staining and biochemical test.

Preparation of consortium: Consortium were prepared by mixing all 3 positive isolates as P1D(P1A+P1B+P1C). A total of three isolates and one consortium were used for degradation of polythene.

Conclusion

In previous study total 20 bacterial organism were isolated from from 3 different dumping yards located in Vishakhapatnam A.P India and out of 20 isolates one bacterium showing more activity in degradation of polythene bags. In current study a total of 15 bacteria were isolated from different sites and after primary and secondary screening three of them showed a positive result and identified as *Staphylococcus sp, Pseudomonas sp, Bacillus sp*. through morphological and biochemical tests. Further according to the degradation pretreated polythene in different interval of time and final weighed of polythene. Isolated P1C (Bacillus *sp.*) showed maximum degradation in 40 days, followed by P1A *(Staphylococcus sp), P1B (Pseudomonas sp.), P1C(Consortium).* Maximum degradation was found to be by *Staphylococcus sp* showed 52% of degradation and *Pseudomonas sp* showed 11% of degradation by weight loss. By observing this result, we can conclude that *Bacillus sp* showed maximum degradation by weight loss of polythene comparing other bacteria. Hence this method can be widely used for degradation of polythene from the environment.

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