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SCREENING OF POLY VINYL CHLORIDE DEGRADING BACTERIA FROM PLASTIC CONTAMINATED AREA OF BADDI

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ABSTRACT
Plastic wastes are posing ever increasing environmental concerns. Recent research works have shown that most of the constituents of plastics can be degraded by microbes and the film plastics can be treated by microbial systems. In this study, poly vinyl chloride (PVC), degrading bacteria's were isolate and characterize from soil dumped with plastics. The PVC degradating bacterial were identified as *E.Coli, Staphylococcus, Pseudomonas* and *Klebsiella*. It was observed that maximum degradation by *Pseudomonas* and degradation after 10 months was 40.53%, 23.06%, 10.92% and 5.32% for *Pseudomonas*, *Klebsiella*, *Staphylococcus* and *E.Coli* respectively.This work concluded that soil contains bacteria's that have ability to carry degradation of poly vinyl chloride (PVC) .

INTRODUCTION

Plastics are artificially made long chain polymeric molecules and come from the Greek word *plastikos* indicating that they are able to form different shapes [1]. Nowadays, plastics are composed of organic and inorganic raw materials comprising Hydrogen, Carbon, Oxygen, Silicon, Nitrogen and chloride. These include plastics such as; polyethylene (PE), polyvinyl chloride (PVC) and many other related polymeric materials. Plastics have been used increasingly due to their attractive combination of stability as well as their balance of thermal and mechanical properties. The synthetic plastics are used in packaging of products like food, medicines, cosmetics, detergents and chemicals. Approximately 30% of the plastics are used worldwide for packaging applications. The utilization is still expanding at a high rate of 12% per annum [1][2]. The increase in production and lack of degradability of commercial plastics, used in packaging industry, has focused attention on environmental concerns. Furthermore, since plastics are typically disposed of in landfills, there are serious concerns about the pile-up of plastic wastes. Recent research works have

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shown that most of the constituents of plastics can be degraded by microbes and the film plastics can be treated by microbial systems [3]. Biodegradation plays a key role in reducing the molecular weight of the polymer by naturally occurring microbes like bacteria, fungi and actinomycetes isolated from different environments [4]. Microorganisms can degrade plastic over 90 genera, from bacteria and fungi, among them; *Bacillus megaterium*, *Pseudomonas* sp., *Azotobacter*, *Ralstonia eutropha*, *Halomonas* sp., etc [5]. Plastic degradation by microbes due to the activity of certain enzymes that cause cleavage of the polymer chains into monomers and oligomers, then the monomers are absorbed into microbial cells and metabolized. Aerobic metabolism produces carbon dioxide and water [6]. Instead of anaerobic metabolism produces carbon dioxide, water, and methane as end products [7]. Hence the main objective of the study was to isolate and characterize poly vinyl chloride (PVC), degrading bacteria from soil dumped with plastics.

MATERIALS AND METHODS

Soil samples

Soil samples were collected from different plastic waste contaminated area of Industrial area baddi (H.P). Soils were collected in polythene bag and used for the isolation of PVC degrading bacteria.

Polyvinyl chloride film preparation

Low molecular weight PVC was used for the preparation of film. The film was prepared by pouring and then evaporating PVC solution in petridish, at 60^0C in hot air oven for 6 hr. The films were sterilized by dipping pit into 70 % ethanol for 15 minute [8].

Preparation of Winogradsky's column

The collected soil samples were placed separately in the glass column. The prepared PVC film was buried in soil sample and to enhance microbial activity in the soil, Bushnell Hass Broth was added in column such that it formed a layer of few inches above the settled soil sample. Columns were sealed tightly to prevent evaporation of water and incubated at room temperature for 6 months [9].

Isolation of Polyvinyl chloride (PVC) degrading bacteria

PVC films were recovered from soil after 6 months. The films were cut in to pieces washed with sterilized water and placed on mineral salt medium (MSM) agar plates. The plates were incubated at 30 ^{0}C. For isolation of bacterial strains loop full of inoculums was taken from MSM agar plate and streaked on nutrient agar plate. Nutrient agar plates were incubated at 30 ^{0}C. To prevent growth of fungi 0.5 % antifungal agent nystatin was added to nutrient agar media [10].

Identification of bacteria:

Identification of the isolates was performed according to their morphological, cultural and biochemical characteristics by following Bergey's manual of systemic bacteriology. All the isolates were subjected to gram staining and specific biochemical test [1]

Biodegradation of plastic in laboratory condition

One gram of sample plastic strips were aseptically transferred to the conical flask containing 50 ml of cultural broth medium, inoculated with different isolated bacterial strain for 10 months. Control was maintained with plastic discs in the microbe-free medium. Different flasks were maintained for each treatment and left in a shaker. After one month of shaking, the plastic discs were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the plastics was calculated [11].

Scanning Electron Microscopy (SEM)

The surface morphology of the PVC film was analysed through SEM. The image of the treated test sample was compared with original untreated sample (control).

Fourier Transform Infra Red (FTIR)

Fourier Transform Infrared Spectroscopy analysis was used for detecting the formation of new functional groups or changes in the amount of existing functional groups. The control and test PVC film pieces were mixed with KBr and made disc, which was fixed the sample holder. A spectrum was taken in triplicate at 400-4000 Cm-1 for each sample.

RESULT AND DISCUSSION

Soil samples, collected from plastic waste contaminated site with heap of solid waste debris from industrial area baddi were used as a source for isolating bacteria having the ability to degrade PVC plastic.A number of bacterial strain were isolated and these strains were tested for having the ability to adhere, grow and degrade PVC plastic. Four bacterial strains were

finally selected on the basis of their good growth, adherence and degradation potential of PVC plastics.

For the identification of bacterial isolate, the morphology of the bacteria on the malt extract media was studied and confirmed by various biochemical tests. Colonies of the strain I were large, thick, greyish white, moist, smooth, opaque or translucent disc. Colonies of strain II were large, rough, shiny, opaque yellow and pigmented, whereas colonies of strain III were surrounded by bluish green coloration. Strain IV was exhibited grayish white mucoid colonies of viscid consistency on nutrient agar.

Identification of selected four bacterial strain was done by gram staining and biochemical test. The strains were identified as *E.Coli*, *Staphylococcus*, *Pseudomonas* and *Klebsiella* (Table 1). Biodegradability of isolated bacteria were examined by exposing PVC films to culture broth medium inoculated with different isolated bacterial strain in a shake flask for 10 months.

Table 1: Characteristics of isolated PVC degrading strain

Colony No	I	II	III	IV
Gram nature	Gram negative rod shape	Gram positive cocci in clusters	Gram negative rod shape	Gram negative rod shape
Catalase test	positive	positive	positive	positive
Gelatine hydrolysis test	positive	negative	negative	negative
Indole test	positive	negative	negative	negative
Methyl Red Test	positive	negative	negative	
Starch hydrolysis test	negative	negative	negative	positive
Citrate Utilisation test	negative	positive	negative	positive
Motility test	positive	negative	positive	negative

Biodegradability of selected bacteria were examined by exposing PVC films to culture broth medium inoculated with different isolated bacterial strain in a shake flask for 10 months.

Table 2 and figure 1 show result of weight loss by different bacterial strain. It was observed that maximum degradation by *Pseudomonas* and degradation after 10 months was 40.53%, 23.06%, 10.92% and 5.32% for *Pseudomonas*, *Klebsiella*, *Staphylococcus* and *E.Coli* respectively.

Table 2: PVC degradation by different bacteria after 10 months

Bacteria	Initial weight(g)	Final weight(g)	Weight loss (g)	Weight Loss (%)
E. Coli	1.0	0.947	0.053	5.30
Staphylococcus	1.0	0.890	0.11	11.00
Pseudomonas	1.0	0.595	0.405	40.50
Klebsiella	1.0	0.769	0.231	23.10

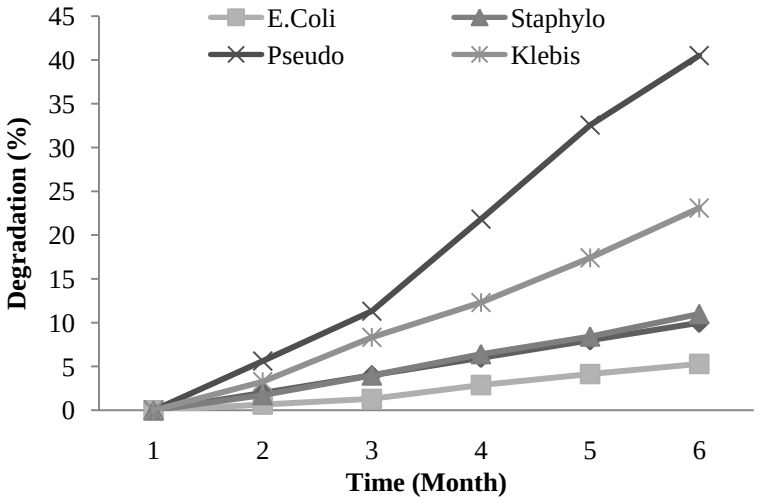

Figure 1: PVC degradation profile of different bacteria

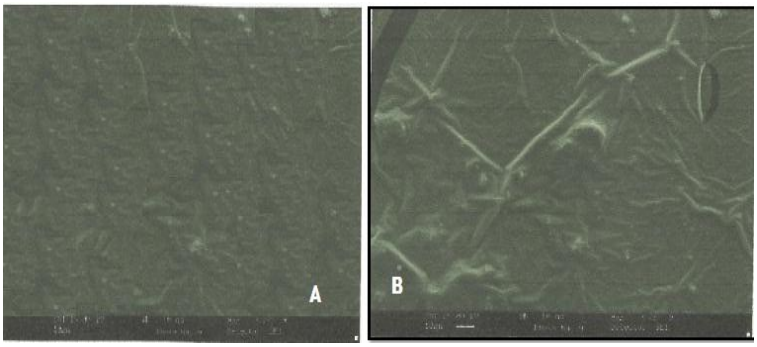

Figure 2: Scanning electronic micrographs of PVC film Untreated (A) and after 10 months with *Pseudomonas* (B)

Unexposed PVC film and film exposed to *Pseudomonas* were examined for SEM analysis (figure 2). There was a significant change on the surface of treated PVC films after biodegradation by *Pseudomonas*. FTIR spectrum of PVC film before and after biodegradation showed (figure 3) the peak at 2370-2375 Cm^{-1} (O-H) and change in intensity of major peak.

It was revealed that chemical and physical changes occurred in PVC film, when exposed to bacteria.

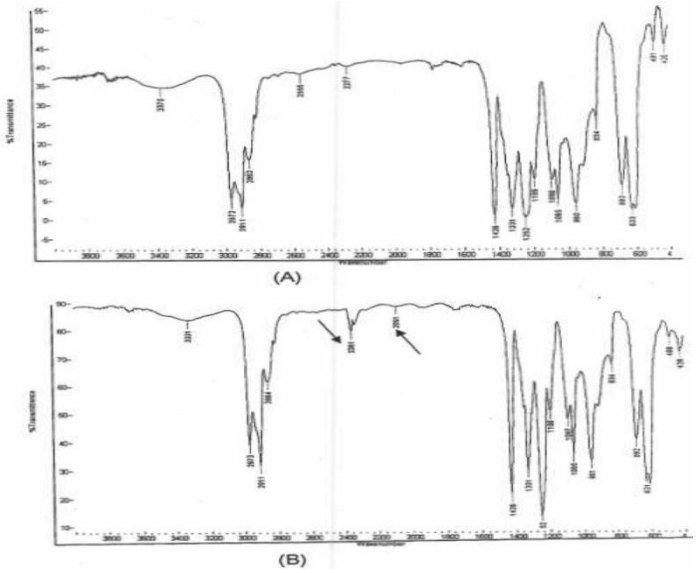

Figure 3: FTIR spectra of PVC film Untreated (A) and after 10 months with *Pseudomonas* (B)

CONCLUSION

From the above study, it is concluded that soil contains bacteria's that have ability to carry degradation of polymers. The four isolated bacterial strain *Pseudomonas*, *Klebsiella*, *Staphylococcus* and *E.Coli* showed better degradation ability, however *Pseudomonas* showed maximum degradation then others. The SEM result showed change in surface structure after biodegradation. The biodegradation of polyvinyl chloride was also confirmed by change in the FTIR spectrum of test sample.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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