



Biodegradation of Plastics by *Pseudomonas putida* isolated from Garden Soil Samples

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ABSTRACT

An attempt was made to isolate *Pseudomonas putida* from garden soil samples and to characterize its degrading ability on plastic material. This work reveals that the garden soil is a good source of microbes capable of degrading plastic materials. *P. putida* have the ability to convert the complex plastic material was determined in terms of weight loss of the material. It degrades the plastic material up to 75.3% within a month. The plastic samples tested in this study were polythene bag, plastic bag, plastic cup and milk cover. Among the plastic samples, milk cover was found to be more degradative (75.3%) plastic material.

Keywords: biodegradation; plastics; *P. putida*; weight loss; intracellular protein.

INTRODUCTION

Plastic is the general term for a wide range of synthetic or semi-synthetic polymerized products. They are composed of organic condensation or addition polymers and may contain other substances to improve performance or economics. About one third of this material is used in the manufacture of disposable items such as wraps, bags and other packaging materials, cups and trays for fast food items and films for agricultural use. The term biodegradable plastics normally refer to an attack by microorganisms on non-water soluble polymer-based materials (Plastics). The extracellular enzymes are too large to penetrate deeply into the polymer material, and so act only on the polymer surface; consequently, the biodegradation of plastics is usually a surface erosion process. Physical disintegration may enhance biodegradation of polymers by increasing surface area for microbial colonization or by reducing molecular weight. A very general estimate of world wide plastic waste generation is annually about 57 million tons [2]. The polythene is the most commonly found non-degradable solid waste that has been recently recognized as a major threat to life. The polythene could sometimes cause blockage in intestine of fish, birds and mammals. Degradation of polythene is a great challenge as the materials are increasingly used. An estimated one million birds and ten thousand marine animals die each year as a result of ingestion of or trapping by plastics in the oceans [11].

Widespread studies on the biodegradation of plastics have been carried out in order to overcome the environmental

problems associated with synthetic plastic waste. Recent work has included studies of the distribution of synthetic polymer-degrading microorganisms in the environment, the isolation of new microorganisms for biodegradation, the discovery of new degradation enzymes, and the cloning of genes for synthetic polymer-degrading enzymes [15] Although photodegradability had been suggested as a solution for plastic litter [14], it has limited practical applications because UV light will not penetrate landfills. Biodegradability of plastics has been proposed as a solution for the waste plastics problem. However none of them is efficiently biodegradable in landfills. For this reason, none of the products has gained widespread use [1]. Hence there is an urgent need to develop efficient microorganisms and these products to solve this global issue [7]. Based on the above circumstances, it was planned to isolate microbes especially the members of the Genus *Pseudomonas* that have the ability to degrade Plastics.

MATERIALS AND METHODS

Collection and processing of the sample

A total of ten soil samples were collected, suspended in sterile distilled water and placed in shaker at room temperature for 24- 48 hours. The serially diluted samples were plated on Trypticase Soy Agar (TSA) and incubated at 37°C for 24 hours. The characteristic isolates were identified by morphological and biochemical identification methods [5]

Screening of plastic degrading strain

The isolated strains were inoculated in mineral salt medium supplemented plastic sample as a sole source of carbon

at a final concentration of 0.1% (w/v) and incubated in rotary shaker at room temperature for 24 hours. After incubation, the turbidity of the medium was checked for growth.

Biodegradation testing

The plastic samples used in this present study were plastic cup (Sample A), polythene bag (B), plastic bag (C), milk cover (D). These plastic samples were collected and carried to laboratory for testing their biodegradability. Each plastic material were tested with isolated strain of *P. putida* and compared with standard strain *P. putida* MTCC 2475.

Pre-treatment of the samples

Heat treatment (70°C) [6] and UV Irradiation (365nm) [3] of the films were carried out by the methods described previously. The films were disinfected chemically by Tween 80 and bleached [8].

Film Culturing and Harvesting

Pre-weighed disinfected films were aseptically added to sterilize basal mineral salt medium. Films in the culture medium were incubated with shaking for 24 hours before inoculation to ensure asepsis. Culture medium was inoculated with pre-enriched standard culture of *P. putida* and isolated pure culture of *P. putida*. It was incubated with shaking at 125 rpm for one month at 37°C. Control was maintained for each type of plastic films in uninoculated mineral salt medium.

At the end of each week, plastic films were harvested, washed with 70% ethanol to remove as much cell mass from the residual film as possible, dried at 45°C for 24 hours.

Methods for Monitoring Biodegradation

Surface changes and Weight loss of plastic materials were determined [4]. The percentage of weight loss of each plastic material used in this study were determined by using the formula,

$$\text{Percentage of weight loss of the material} = \frac{\text{Weight loss of the sample}}{\text{Original weight of sample}} \times 100$$

RESULTS AND DISCUSSION

Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics. A total of ten soil samples were collected from various garden in and around Chennai. The collected samples were processed for the isolation of *P. putida*. The efficiency of degradation of isolated strains and standard strain were compared. Four isolates were isolated on Trypticase Soy Agar (TSA) medium. The isolated strains were identified as *P. putida* by performing appropriate identification tests. The results of various staining, physiological, and biochemical characteristics of standard strain *P. putida* MTCC 2475 and isolated strains were shown in Table 1. The isolated strains were confirmed as *P. putida* by absence of gelatin hydrolysis test and absence of growth on Cetrimide agar.

Biodegradation test

Surface changes in plastic sample

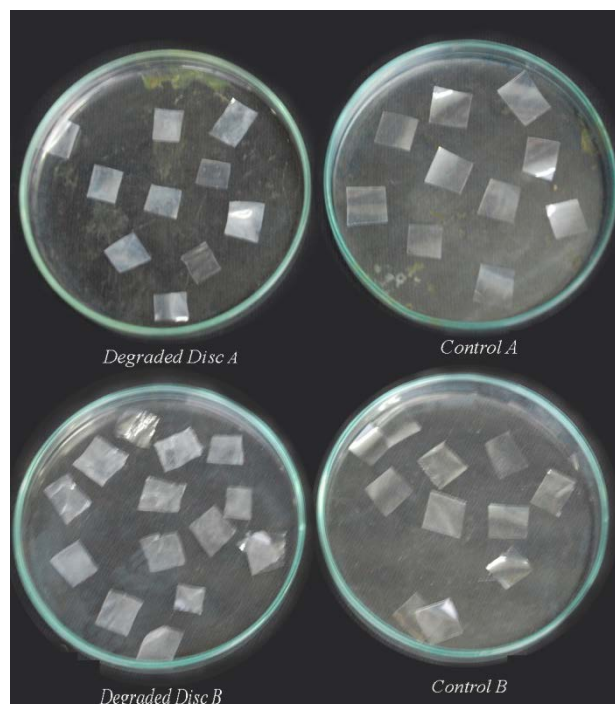
In most applications envisaged for films or fibers in contact with the soil, loss in tensile properties and weight loss are the most relevant practical criterions to determine its degradation [10]. The various surface changes of the plastic samples were observed after incubation with soil isolates. The surface of the

plastic samples has turned from smooth to rough with cracking which are shown in Fig. 1.

Table 1: Identification and characterization of *P. putida* isolated from garden soil samples

Test for identification	<i>P. putida</i> MTCC 2475	Isolated strains
Gram staining	-	-
Motility test	Motile	Motile
Catalase test	+ ^a	+
Oxidase test	+	+
Oxidative-fermentative test	Oxidative	Oxidative
Glucose test	- ^b	-
Sucrose test	-	-
Mannitol test	-	-
Lactose test	---± ^c	---+
IMViC	K ^d / K, no gas	K / K, no gas &
Triple sugar iron agar test	& no H ₂ S ^e	no H ₂ S
Urease test	+	+
Nitrate reduction test	-	-
Gelatin hydrolysis test	-	-
Cetrimide resistance test	+	+
Growth at 28°C	-	-
Arginine dihydrolase	-	-
Lysine decarboxylase	-	-

^a(+), Positive; ^b(-), Negative; ^c(±), Positive or negative; ^d(K), Alkaline; ^eH₂S, Hydrogen sulfite production.



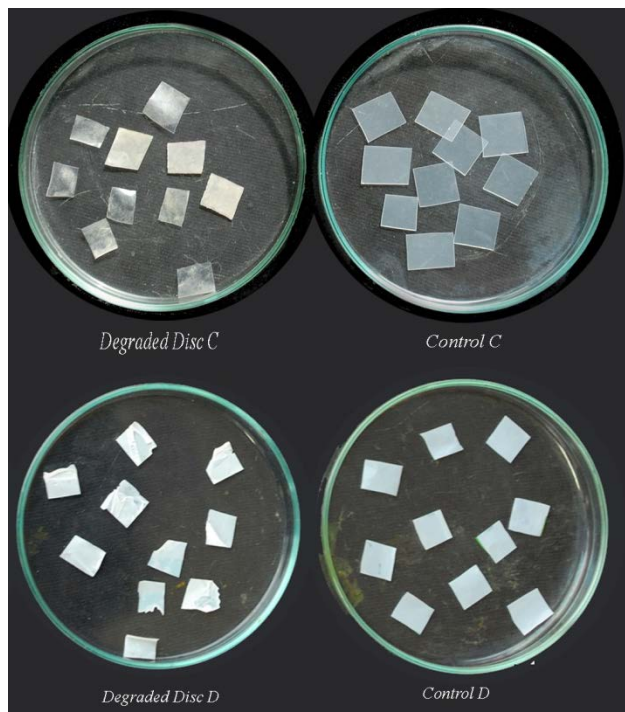


Fig. 1: Surface changes in the plastic sample - Plastic cup (A), Polythene bag (B), Plastic bag (C) and Milk cover (D)

Weight loss of plastic sample

Fig. 2 shows the rate of degradation (per month) of plastic samples inoculated with isolated strains and standard strain. Among the samples used for this study, Plastic sample D was found to be more degradative in the range of 63.1% to 75.3%. Among the four isolated strains, plastic sample D was highly degraded by strain 1 and strain 2. The remaining strains were degrade the sample similar to standard strain used. The strain 1 and 2 showed the levels of biodegradation were high 75.3% and 71.7% compared with standard strain used 63.1%. Plastic degrade abilities of the bacteria ranged from 4-17% [9]. Surface morphology of polyethylene/starch film has been analyzed by scanning electron microscopy (SEM) before and after degradation. Physico - mechanical properties has also been determined before and after degradation of film in order to understand the rate as well as the mechanism of degradation [12]

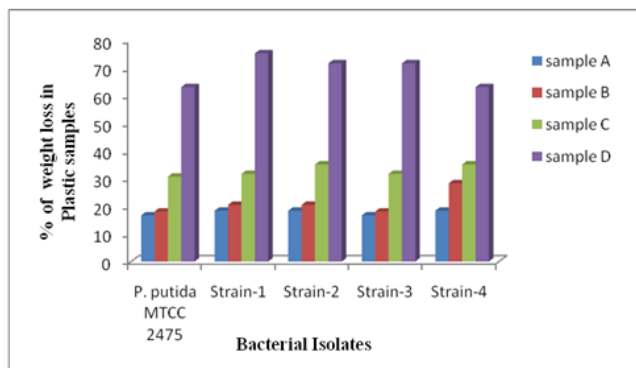


Fig. 2 Degradation of plastic samples under shake flask culture - % of weight loss of plastic samples taken

P. putida has the ability to tolerate and degrade many toxic and hard polymers substances [5]. It has experimentally been proved that *P. putida* cause degradation of all plastic samples used for this study such as A, B, C and D within a month. Among the samples, D (milk cover) was found to be more degradative up to 75.3%. Hence, *P. putida* are efficient in biodegradation of plastic materials. The mechanism of degradation is not known. The surface of plastic materials has turned from smooth to rough with cracking. This may due to the compounds secreted extracellularly by the microbes that may break the complex molecular structure of plastics [13]. Hence, further study on microbial enzymes or organic acids in degradation of the polyethylene plastics will pave way for finding technology for degrading the plastic materials, which are otherwise hazardous to environment. Therefore, the current study reveals the *P. putida* were found to be efficient bacteria for bioremediation of plastic material.

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