International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 20-08-2023, Accepted: 05-09-2023, Published: 21-09-2023
Volume 5, Issue 3, 2023, Page No. 67-69

Isolation and characterization of polivinyl chloride (Pvc) degrading bacteria from soil sample of plastic dump site

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Abstract
The use of Petroleum-based plastics has steadily increased which leads to a significant environmental problem. In the current study, bacteria were isolated from the soil samples of 4 different plastic waste dump sites of Darjeeling. A total of 943 bacteria were isolated from the soil samples by using standard microbiological procedures. The isolates were characterized by various morphological and biochemical tests. After the identification of isolates, 20 isolates were selected for their examination for plastic degradation ability. Isolates were studied for their PVC degrading ability in terms of weight loss percentage. The bacterial isolates were identified as *Streptococcus* (7), *Micrococcus* (6), *Staphylococcus* (5) and *Bacillus* (2). These isolates were checked for their ability of plastic degradation. Biodegradation of the plastic was checked in nutrient medium. The three bacterial isolates that showed degradation of PVC strips were *Staphylococcus aureus*, and two strains of *Streptococcus*. These isolates decreased the weight of plastics by 4mg, 2mg and 4mg respectively.

Keywords: Plastic degrading bacteria, soil isolates, PVC, Darjeeling

Introduction

The invention and commercialization of Petroleum-based plastic production have made man's life easier in many aspects due to its durability, water resistance, and other characteristics. The term plastics are referred to larger sized plastic materials ranging from 2.5 cm to 1 m of known items, such as bottles, large containers, plastic toys, and buoys (Young AM *et al*., 2016) [15]. The production of plastics is increasing day by day which further increases the number of left-over plastics into the environment which possess a big threat to each and every part of ecosystem.

The massive use of plastics especially in the medicinal, industrial area, commercial, and municipal field, has led to a significant environmental problem. Plastics are also used in packaging of food, detergents and cosmetics (Thakur, 2012; Elahi *et al*., 2021) [10, 5]. In 2015, Global petroleum-based plastic production reached 322 million tons, while in 1950, the production was 1.7 million tons (Plastics Europe, 2017).

Petroleum based plastics may be fragmented, but cannot break down naturally due to their profound stability (Environmental Investigation Agency, 2016). Even the degradable plastic materials can last for many years, depending on environmental circumstances, such as temperature, oxygen, and the exposure levels of ultra violet light (Hopewell J *et al*., 2009) [6]. Stabilizers and plasticizers are also included to enhance the characteristics features and efficiency of petroleum-based plastic materials. (Thompson RC *et al*., 2009). And, during its degradation process, they may be leaked into the environmental system (Teuten EL, S *et al*.,2009) [13].

Environmental pollution by plastic waste was first reported in the 1970s (carpenter and smith, 1972) [3]. Non-degradable plastics, typically known as synthetic plastics, are derived from 7 petrochemicals and are higher in molecular weight due to the repetitions of small monomer units (Imre and Pukánszky, 2013; elahi *et al*., 2021) [5].

Polyvinyl chloride (PVC) is a synthetic resin made by polymerization of vinyl 54 chloride monomer (VCM), developed in 1926 by Waldo Aemon and the
B.F. 55 Goodrich company. This material is the third-most widely-produced plastic in the world, after polyethylene and polypropylene, with 37.3 million tons consumed globally in 2014 and estimated to reach 50.27 million tons by 2020 (business wire, 2016, tang *et al*., 2018) [2, 9]. Biodegradation of plastics such as PVC involves at least three reactions which includes depolymerization of polymer chains, Formation of oxidized intermediates, and mineralization of intermediates to CO_2, H_2O and Cl- when PVC is degradation target (Peng *et al*., 2020). In recent years various microbes have studied on their PVC degrading abilities. Microorganisms such as *Pseudomonas citronellolis, Bacillus flexus* (Giacomucci *et al*., 2019), *Micrococcus sp.* (Yadav *et al*., 2021) and Klebsiella *sp.* (Zhang *et al*., 2022) have shown PVC degradation.

Even in Darjeeling town the use of plastic has increased profoundly. The town witnesses the plastic waste dumping sites in and around. Therefore, the current study envisages the study of plastic degrading bacteria from Darjeeling.

Methodology

1. Sample collection

a. Soil sample collection

Soil samples with plastic waste were gathered from 4 different dump sites of Darjeeling. Darjeeling is a town situated in the West Bengal state of India and is located in the Eastern Himalayas. The samples collecting sites were as follows: a. College area, b. Singamari, c. Frymall village, and d. Near Padmaja Naidu Himalayan Zoological Park. The samples were collected in a sterile polythene pack in an aseptic manner and were taken into laboratory (Collee JG *et al*., 1996) [4].

b. Polymer sample collection

The Poly Vinyl Chloride (PVC) film was obtained from hardware shop of local market of Darjeeling.

2. Isolation of bacteria

1 gram of homogenized soil sample was transferred into a conical flask having 99ml autoclaved sodium chloride solution. Then it was kept in shaker for 1hour to create the suspension. Then, it was left undisturbed for 15 min so that bigger particles can settle down. This mixture was serially diluted up to 10-6 dilution in 0.9% saline. An amount of 1 ml of each soil sample from selected dilutions (usually1: 100 and 1:1000) were taken and poured using pour plate method on sterile nutrient agar petri plates (Aneja kr., 2007, Powthang *et al*., 2017) [1]. The petriplates were incubated at 37ºC for 24 hours and the following day the isolates were identified on the basis of Gram staining, colony morphology and standard biochemical tests (Collee JG *et al*., 1996) [4].

3. Pre-treatment of PVC strips

PVC strips were cut into 1×1-cm and weighted. Then those were cleaned under tap water followed by wash with ethanol. The strips were again washed with distilled water. Then, the strips were kept in 0.1% mercuric chloride. And at last, the strips were washed with distilled water and air dried in laminar air flow (Usha, *et al*. 2011, Singh *et al*. 2016).

4. Degradation of pre- treated PVC

Initially weighed strips of 1×1-cm size of PVC strips werer aseptically transferred to the conical flask containing 50 ml of nutrient broth medium. Then, the identified soil isolates were inoculated into those conical flasks. Control flask was also maintained with PVC film in the microbe-free medium. These conical flasks were incubated into a shaking incubator at 37˚C. After every week, the polythene strips were collected from the flask kept in shaking incubator aseptically, washed thoroughly using distilled water, shade-dried and then weighed to measure the final weight and to calculate the percentage weight loss using the below mentioned formula. (Usha, *et al*. 2011, Singh *et al*. 2016).

$$\text{Weight loss \%} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Results and discussion

1. Isolation and identification of bacteria

In the current study, bacteria were isolated from the soil samples of 4 different plastic waste dump sites of Darjeeling. A total of 943 bacteria were isolated from the soil samples by using standard microbiological procedures.

The isolates were characterized by various morphological and biochemical test. After the identification of isolates, 20 were selected for their examination for plastic degradation ability. The 20 isolates which were identified as *Streptococcus* (7) followed by *Micrococcus* (6), *Staphylococcus* (5) and *Bacillus* (2). Isolates were studied for their PVC degrading ability in terms of weight loss percentage.

Table 1: Viable number of colonies obtained from a soil sample

Sources	Dilutions	Colonies	Cfu count
Dump site 1 College area	10-3	95	5300000 cells/g
	10-4	75	
	10-5	53	
	10-6	18	
Dump site 2 Singamari	10-3	115	6000000 cells/g
	10-4	76	
	10-5	60	
	10-6	21	
Dump site 3 Frymall village	10-3	95	250000 cells/g
	10-4	68	
	10-5	25	
	10-6	8	
Dump site 4 Near Padmaja Naidu Himalayan Zoological park	10-3	128	2200000 cells/g
	10-4	78	
	10-5	22	
	10-6	6	

Colony forming unit is counted by following method:

$$\text{Viable cells/g dry soil} = \frac{\text{mean plate countx dilution factor}}{\text{dry weight of soil}}$$

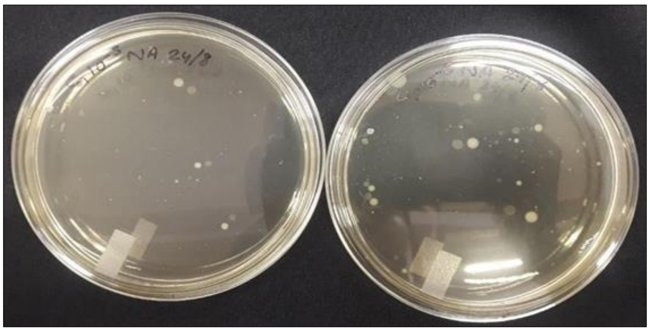

Fig 1: Isolation of bacteria from soil of 10-5 and 10-6

2. PVC strip degradation

After every week of incubation, the weight of the plastic strips was calculated. No reduction in the size of the plastic was observed until 8 weeks. After 8 weeks reduction in the weight of the plastic was observed. The three bacterial isolates that showed degradation of PVC strips were *Staplylococcus aureus*, and two strains of *Streptococcus*. These isolates decreased the weight of plastics by 4mg, 2mg and 4mg respectively.

Table 2: Degradation of PVC strips by soil isolates after 8 weeks

Isolates	Initial weight (mg)	Final weight(mg)	Difference in the weight	Weight loss/month (in%)
Staplylococcus aureus	200	196	4	2
Streptococcus sp.	186	184	2	1.075
Streptococcus sp.	203	199	4	2

Discussion and conclusion

In the current study, the soil isolates like *Microcossus sp*., and *Bacillus* did not show any weight loss of PVC strips. However, in a study by Giacomucci *et al*, in 2019 PVC degradation was observed by *Pseudomonas citronellolis*, and *Bacillus flexus*. On the contrary, Patil *et al*, 2012 revealed that *Micrococcus* sp. showed 0.36% release of chloride and 8.87% mineralization measured in terms of carbon dioxide evolution respectively over a period of 70 days.

Moreover, Kumar *et al*., identified PVC degrading bacteria as *Pseudomonas, Klebsiella, Staphylococcus* and *E. coli* with the degradation ability of 40.53%, 23.06%, 10.92% and 5.32% respectively in a duration of 10 months.

Microbial degradation of a solid polymer like polyethylene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities. Development of multicellular microbial communities known as biofilm attaches to the surface of synthetic wastes have been found to be powerful degrading agents in nature. Microbial degradation of plastic has shown an eco-friendly way to solve this problem. The removal of microplastics, their toxicity and the utilization of microbes remain to be addressed. Massive research studies are required for screening and isolation of microbial species which can effectively degrade and decompose various plastic polymers. The implementation of microbial methods for plastic degradation can open newer avenues and help reduce the global problem of plastic waste management.

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