Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
CODEN: JPCBBG
Impact Factor (GIF): 0.701
Impact Factor (SJIF): 3.905
March - May 2018; 6(1):62-67
Published on: May 21, 2018

Research Article

Biodegradation of PEG and Polythene Bag using PGPR Isolated from the Rhizosphere of *Celosia cristata* L

Sunanda Dutta, Avishek Sarkar, Sikha Dutta*

Applied Mycology and Molecular Plant Pathology Laboratory, CAS Dept. of Botany, University of Burdwan, Burdwan-713104, West Bengal, India

***Corresponding Author:** Sikha Dutta, Applied Mycology and Molecular Plant Pathology Laboratory, CAS Dept. of Botany, University of Burdwan, Burdwan-713104, West Bengal, India

Received: 15 March 2018 **Revised:** 09 April 2018 **Accepted:** 12 April 2018

ABSTRACT

The major environmental threat is the slightest pace of degradation or non-biodegradability of the organic materials under natural clause, e.g. plastics. The plastics of various forms such as nylon, polycarbonate, polyethylene-terephthalate, polyethylene, polypropylene, polystyrene, polytetrafluro ethylene, polyurethane and polyvinyl chloride (Smith, 1964) are being endlessly used in our daily life. The global utility of polyethylene is escalating at a velocity of 12% per annum. In recent times, the biodegradation of plastic waste and the use of microorganisms to demean the polymers have gained remarkable magnitude because of the inefficiency of the chemical and physical disposal methods, and the ecological harms they cause. The present work that we have done evaluates the biodegradation efficacy of the isolated bacteria from the rhizospheric soil of *Celosia cristata* against two different microns of plastic bags (40 and 150 microns) and with different concentrations (5-25%) of Poly-Ethelene-Glycol. The pure culture of the bacterial isolate (SD/B) was then subsequently identified by using 16s rDNA sequencing and subjected to assay its polythene degrading property if any. The bacteria were identified to be *Pseudomonas aeuriginosa*. Overall study showed that the bacteria reveals a considerable polythene degradation capability and in near future it can be used to reduce the amount of plastic waste that has been hastily accumulating in the nature, in a cheap and eco-friendly method.

Keyword: Biodegradation; microbes; PEG (polyethylene glycol); polythene

INTRODUCTION

Polythene is the most frequently found non-degradable solid dissipate that has been identified as a great danger to life in recent times. It has an extensive array of applications in our everyday use because of its easy processing for various goods used in carrying food products, wrapping textiles, manufacturing laboratory instruments and automotive components [1]. Polythene constitutes 64% of the total synthetic plastic as it is being used in huge amount for the craft of bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes etc. [11]. Polythene bags are made of polyethylene. The synthetic polymers are highly hydrophobic in nature and have a high molecular weight. Annually 500 billion to 1 trillion polythene bags

are being used habitually all over the world. Approximately 140 million tons of synthetic polymers are produced worldwide each year [15; 24] and this plastic pollution is caused due to inappropriate recycling and waste managing systems [8]. With such huge amount of polyethylene getting accumulated in the environment and their dumping evoke a big ecological concern. To the marine life polythene waste is documented as a chief hazard. Occasionally, it is responsible for the intestinal blockage in the fish, birds and other mammals [20; 17; 4]. As a result of plastic infectivity in the marine environment minimum 267 species has been affected, which includes all mammals, sea turtles (86%) and seabirds (44%) [3]. The fatality of other terrestrial animals such as cow was also reported due to polythene intake [18]. Commonly used packaging plastic (mainly polythene) constitutes about 10% of the total municipal waste generated around the earth [2]. Only a fraction of this polythene waste is recycled while most of the wastes go into the landfills and take hundreds of years to degrade [10; 13]. Use of polythene is rising every day and its degradation is becoming a great confront.

One of the major groups of synthetic polymers is the polyethylene glycol (PEG) group. PEG is used in pharmaceuticals, tobacco, beer, antifreeze for automobile radiators, and special lubricants for the textile industry. Possibly the biggest use of PEG is in the producion of nonionic surfactants, and PEGs of various molecular weights are linked with phenyl or other aromatic moieties to produce surface-active agents. Among 20 to 25% of all surfactants, hold some form of PEG [22].It was found that the pace and degree of biodegradation was said to decline with increasing chain length and PEGs with a molecular weight (above 500) apparently were being non-biodegradable [14], hence concern has been expressed in recent years regarding the fate of these polymers released to the environment.

The method of Biodegradation being cheap and eco-friendly, is an approaching trend in this field of degradation [6]. Which includes mostly microorganisms that can degrade the polythene. The microbial degradation of plastic is carried out by enzymatic activities which direct to the breakdown of polymer into monomers and oligomers followed by metabolism of microbial cells. Aerobic metabolism leads to the formation of carbon dioxide and water [21] and on the contrary anaerobic metabolism yields carbon dioxide, water and methane as end products [6]. Considering this the present work was undertaken to isolate the bacteria from the rhizosphere of the medicinal plant *Celosia cristata* L. to investigate its polythene as well as synthetic polymer degrading property if any.

MATERIAL METHODS

Collection of soil sample

The soil was collected directly by uprooting the plant. Soil sample of about 10 gm was collected from the root region (rhizosphere) of Celosia *cristata* L. and put into polythene bags, labeled and stored at 4°C until analysis.

Isolation and Identification of the rhizobacteria

Serial dilution of the collected rhizospheric soil samples were then done upto the level of 10^{-6} dilution.

Bacterial colonies were isolated on Nutrient agar medium. For the study of colony characteristics the bacterias were grown into the plates. The plates were inoculated by spreading 0.1 ml of a 22 hours old culture on the surface by a glass spreader and incubated at 30±2°C. The colony characters were deliberated after 48 hours of interval.

For identification purpose the isolates were then sent to MCC for identification 16S rDNA sequencing and general strain deposition.

Study of PEG bio-degrading property

For the study of PEG bio-degradation property by the isolate, the microbes were inoculated into Nutrient broth media having different concentrations of PEG-6000; viz.,- 5%(0.146 M Pa), 10%(0.292 M Pa), 15%(0.438 M Pa), 20%(0.584 M Pa) up to 25%(0.73 M Pa) and incubated at 30±2°C for 3-4 days and the growth was measured spectro-photometrically at 540nm of web length.

Study of Polythene Bag bio-degrading property

At first weighed strips of 3×3-cm size of 40 and 150 micron polythene were aseptically transferred after surface sterilization with ethanol, to the conical flask containing 50 ml of nutrient broth medium and inoculated with

bacteria (0.5ml). Control was maintained with the plastic strips in the microbe-free medium. Different flasks were then kept in the shaker for 10, 20 and 30 days in that order. Subsequent to the respective period of trembling, the polythene strips were collected and washed meticulously using distilled water, shade-dried and then weighed for checking the final weight and fraction of weight loss was checked using the following formula. [23; 19].

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

RESULTS

After isolation and pure culture establishment, the bacterial isolates were sent to MCC for identification and general deposit and identified as *Pseudomonas aeuriginosa;* strain accession number MCC-3198.

Sequence Text (in FASTA format)

AGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATG
AAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGT
GGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATC
TTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACC
AAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAG
ACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCC
GCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAA
TACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGT
AATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAA
GTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGG
TAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGC
GAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTG
GCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC
TGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTG
CTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCT
TGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCG
GTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGC
AGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGATCAGAATGTCACGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAA

Summary of the closest neighbour(s) the sample

Sl. No.	Closest Neighbour	Strain	Citation	Accession No.	Pairwise Similarity (%)	Diff/ Total nt
1.	*Pseudomonas aeruginosa*	JCM 5962(T)	(Schroeter 1872) Migula 1900	BAMA01000316	100	0/1421

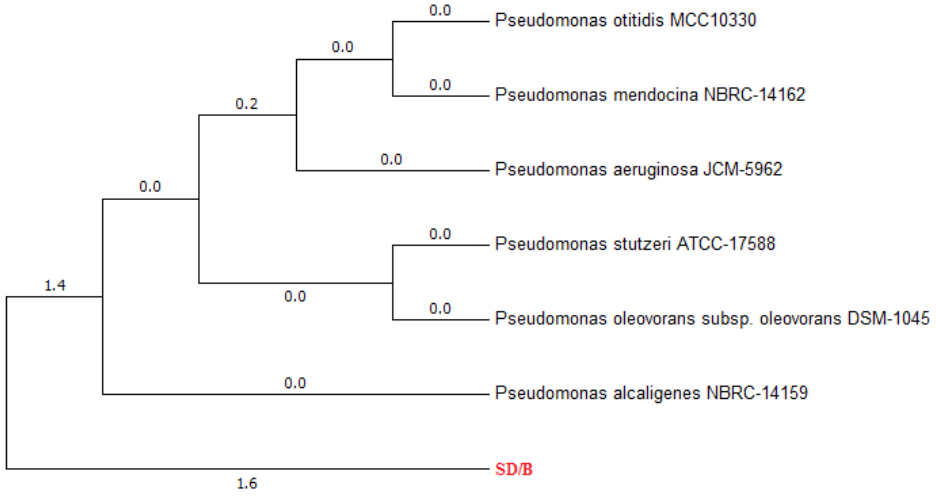

Fig.1: Phyllogenetic Tree Of Sd/B

Table 1: Growth of the isolated bacteria in respective to different concentration of PEG

Concentration of PEG	Atmospheric Pressure Produced by PEG in Mpa Unit	O.D. at 540nm of wavelength
5%	0.146	0.926 ± 0.004
10%	0.292	0.208 ±0.016
15%	0.438	0.188 ± 0.008
20%	0.584	0.141 ± 0.011
25%	0.730	0.125 ± 0.019

It has been found that though the growth rate of the bacteria decreases in respective of the increasing concentration of PEG but it has never gone down to zero; this indicates that the bacteria can not only tolerate the increasing concentration of PEG but also it can degrade the PEG to some extent.

Table 2: Biodegradation of two different microns of plastic by the bacteria at different days of interval

Days of Interval	40 Micron Plastic			150 Micron Plastic		
	Initial Weight (gm)	Final Weight (gm)	% of Weight Loss	Initial Weight (gm)	Final Weight (gm)	% of Weight Loss
10	1.0	0.910 ± 0.004	9 ± 0.4	1.0	0.972 ± 0.005	2.8 ± 0.5
20	1.0	0.891 ± 0.016	10.9 ± 1.6	1.0	0.968 ± 0.003	3.2 ± 0.3
30	1.0	0.876 ± 0.019	12.4 ± 1.9	1.0	0.937 ± 0.011	6.3 ± 1.1

From the above table it clearly indicates that the bacteria surely degrades plastic but the degradation rate of the plastic having lesser thickness is found to be higher than that of the thicker one.

a **b** **c**

Fig 2: Plastic inoculated with bacteria at 10 days of interval (a. 150 micron and 40 micron from left to right respectively); Plastic inoculated with bacteria at 20 days of interval (b. 150 micron and 40 micron from left to right respectively); Plastic inoculated with bacteria at 30 days of interval (c. 150 micron and 40 micron from left to right respectively)

DISCUSSION

The microbes are the main tool in bio-degradation of matters including synthetic products and this is controlled by the exo and endo-enzymes produced by them. These enzymes help to break down the complex polymers into shorter chains and again in turn used as carbon and energy sources for those microbes [5; 7]. The bacterial species that have been reported to have significant biodegrading capacity include *Bacillus* sp., *Klebsiella* sp., *Rhodococcus* sp., *Escherechia* sp., *Azotobactor* sp., *Flavobacterium* sp. etc. [16] and *Desulfotomaculum nigrificans*, *Pseudomonas alcaligenes* [12].

In current study we have included two different microns of polythene bag viz., 40 microns and 150 microns; and a synthetic polymer PEG-6000 of different concentrations (5-25%). It has been found that the polythene with lower density has an elevated degradation rate than that of high density polythene bag. It's also been found that the degradation rate of PEG-6000 also decreases with the increasing concentration of PEG, but it never turns to zero even at the highest possible atmospheric pressure level i.e., 0.73 Mpa unit.

By observing these results we can conclude that the test bacterium SD/B has the potential to degrade the synthetic polymers, hence it can be used as a promising tool for the elimination of synthetic polymers from the environment.

REFERENCES

1. Arutchelvi J et al. Biodegradation of polyethylene and polypropylene. Indian J Biotechnol 2008; 7: 9-22.
2. Barnes D K A et al. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B Biol Sci 2009; 364:1985-1998.
3. Coe J M, Rogers D B. Marine Debris: sources, impacts, and solutions. Science. New York: Springer; 1997.
4. Denuncio P, Bastida R, Dassis M, Giardino G, Gerpe M. Plastic ingestion in Franciscana dolphins, Pontoporia blainvillei (Gervais and d Orbigny, 1844). eds Argentina. Mar Pollut Bull 2011; 25-30.
5. Frazer A C. O-methylation and other transformations of aromatic compounds by acetogenic bacteria. eds. Acetogenesis. New York: Chapman & Hall, Drake H L. 1994; p 445.
6. Gu J D, Ford T E, Mitton D B,Mitchell R. Microbial degradation and deterioration of polymeric materials. eds The Uhlig Corrosion Handbook (2nd Edition). New York: Wiley; 2000, p 439.
7. Hamilton J D et al. Polymers as solid waste in municipal landfills. J Air Waste Manage Assoc 1995; 43: 247-251.
8. Jayasiri H B et al. Plastic litter accumulation on high-water strandline of urban beaches in Mumbai, India Environ Monit Assess 2013; 185: 7709-7719.
9. Kim et al. Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 2012; 62: 716-721.
10. Lederberg J. Encyclopedia of microbiology D-K. New York: Academic Press; 2000.
11. Lee B et al. Biodegradation of degradable plastic polyethylene by Phanerochaete and Streptomyces species. Appl Environ Microbiol 1991; 57: 678-685.
12. M. Ariba Begum et al. Biodegradation of polythene bag using bacteria isolated from

soil. Int J Curr Microbiol App Sci 2015; 4(11): 674-680.
13. Moore CJ. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ Res 2008; 108: 131-139.
14. Patterson S J et al. Nonionic detergent degradation. HI. Initial mechanism of the degradation. J Am Oil Chem Soc 1970; 47:37-41.
15. Roy P K et al. Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. Polym Degrad Stab 2008; 93:1917-1922.
16. Sangale M K et al. A review on biodegradation of polythene: The microbial approach. J Bioremed Biodegrad 2012; 3: 164.
17. Secchi E R, Zarzur S. Plastic debris ingested by a Blainville s beaked whale, *Mesoplodon densirostris*, washed ashore in Brazil. Aquatic Mammals 1999; 25: 21-24.
18. Singh B. Harmful effect of plastic in animals. Indian Cow 2005; 2:10-18.
19. Singh G et al. Biodegradation of Polythenes By Bacteria Isolated From Soil. Int J Res Develop Pharm Life Sci 2016; 5(2):2056-2062.
20. Spear L B et al. Incidence of plastic in seabirds from the tropical pacific l984-1991: Relation with distribution of species, sex, age, season, year and body weight. Mar Environ Res l995; 40: 123-146.
21. Starnecker A, Menner M. Assessment of biodegradability of plastics under stimulated composting conditions in a laboratory test system. Int Biodeterior Biodegrad 1996; 85-92.
22. Swisher R D. Surfactant biodegradation. New York: Marcel Dekker Inc.; 1970.
23. Usha R et al. Screening of polyethylene degrading microorganisms from garbage soil. Libyan Agri Res Center J Int 201; 2(4): 200-204.
24. Vatsel D, Anbuselvi S. Isolation and characterization of polythene degrading bacteria from polythene dumped garbage. Int J Pharm Sci Rev Res 2014; 25(2): 205-206.

Cite this article as:
Sunanda Dutta, Avishek Sarkar, Sikha Dutta. Biodegradation of PEG and Polythene Bag using PGPR Isolated from the Rhizosphere of *Celosia cristata* L. J Pharm Chem Biol Sci 2018; 6(1):62-67