RESEARCH ARTICLE

Isolation, Phylogenetics and Growth response of Low Density Polyethylene (LDPE) degrading bacteria

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ABSTRACT

Urbanization, changes in life style and developmental activities have led to tremendous generation of plastic waste across India amounting to 1.5 million tonnes per annum creating the so called "white pollution". Municipal solid waste in India contains 1-4% by weight of plastic waste mainly consisting of low density polyethylene (LDPE) requiring its efficient disposal. In view of this, the current work is centered on bioremediation (biological cleanup) of plastic waste as a method of treatment, which is generally cheaper and more environment friendly than other alternatives such as incineration, chemical treatment or landfills.

Soil samples from various garbage dumping grounds and parks were used for isolation of LDPE degraders by enrichment culture technique using Bushnell & Haas Broth containing increasing concentrations of LDPE (0.2-1.0 %) as sole source of carbon. Eighteen bacterial isolates obtained after five rounds of enrichment were identified using 16s rDNA sequencing. Isolates showing good growth response to LDPE were found to belong to genus *Pseudomonas* and *Arthrobacter*. These five isolates were further evaluated for their ability to degrade LDPE on the basis of growth response to LDPE in liquid media, % weight loss by gravimetric analysis and reduction in molecular weight by GPC.

Keywords: Plastic, LDPE, Polyethylene, bioremediation, phylogenetic.

INTRODUCTION

The word plastic comes from Greek word "plastikos", which means 'able to be moulded into different shapes' (Joel, 1995). The development of plastics in the world started around 1930 with the introduction of polystyrene, polyvinyl chloride, polyethylene, nylons, and with transition from coal-based to petrol-based chemicals. The condensation and addition polymers came into market since 1950s (Datta *et al.*, 1998). Originally, plastics were mimicking and replacing natural products like lacquer, shellac, amber, etc. But today, they are largely synthetic

materials made from an extremely inexpensive but non-renewable resource, crude oil (APME, 1999). Thus, plastics have become technologically significant and they have come to replace glass, wood, masonry and other constructional materials, and even metals in many industries (Cain, 1992). Ironically, these same properties are proving to be a major environmental problem when these materials enter into the waste stream. Because plastics are designed to resist degradation, they can become permanent residents in landfills.

Every year more than 140 tonnes of plastic is produced worldwide. As conventional plastics are persistent in environment, improperly disposed plastic materials are significant source of environment pollution, potentially-harming life (Nir *et al*., 1993). Various improper methods of disposal include burying or burning of plastic materials which releases harmful or toxic pollutants into the environment thereby endangering the biosphere. The burning of PVC plastics produce persistent organic pollutants known as 'furans' and 'dioxins'. These pollutants are known to cause adverse effects in humans, including immune and enzyme disorders and chloroacne, and they are classified as possible human carcinogens. Health may be affected by polymer itself, by chemicals added to the plastics to make it more flexible stable and flame retardant or colouring agents (Jayasekara *et al*., 2005). Littering not only threatens wildlife and marine life, but also cause considerable aesthetic nuisance (Yabannavar and Bartha, 1994). They have a direct impact on marine ecosystems and are believed to be responsible for the death of a very large number of birds and fish by ingestion or strangulation (Scott, 1990).

Since, plastics have become an integral part of our everyday life, it is impossible to prevent even in part, the release of these materials into the environment, consequently, it is important to discover the ways to biodegrade these compounds (Cacciari *et al*, 1993). In view of this, most of the current work is centered on bioremediation of plastic waste as a method of treating plastic waste, which is generally cheaper and more environment friendly than other alternatives such as incineration, chemical treatment or landfills. From recent work, it has been concluded that microorganisms capable of degrading polymer components might play a very important role in degradation of plastics (Ishigaki *et al*, 2000).

MATERIALS & METHODS:

(1) Enrichment & Isolation of LDPE degraders:

Materials:

Sterile Busnell and Haas Broth (Bushnell and Haas, 1941) with polyethylene as sole carbon source, Soil samples collected from various garbage dumping grounds and parks (Gorai Dumping Ground, Deonar Dumping Ground, Borivli National Park, Mahim National Park, Bombay Port Trust garden), Sterile nutrient agar.

Method:

5g samples of soils were collected from various garbage dumping grounds and parks showing visible littering of plastic and were inoculated in 100 mL of sterile Busnell and Haas Broth containing increasing concentrations of polyethylene (0.2-1.0 %) as sole source of carbon (Hadad *et al.,* 2005). Five successive rounds of enrichment were carried out by incubating the flasks at 28±2°C for one month under shaking conditions at 120 rpm. Individual isolates were obtained by streaking the enriched medium on sterile Nutrient agar after 48 h incubation at 28±2°C

(2) Identification of the isolated bacterial strains by 16s rRNA:

Materials:

Nucleotide BLAST (NCBI site),
Clustal omega program on the internet (http://www.ebi.ac.uk/Tools/msa/clustalo/)

Methods:

Total genomic DNA for 16S rDNA amplification was isolated from cells grown to the late exponential phase. Amplification of the 5' end of the 16S rRNA gene was performed with universal primers and the 16s rDNA sequences were obtained. The 16s rDNA sequences thus obtained were aligned against known deposited 16S rDNA sequences using nucleotide BLAST on NCBI website. Determination of possible phylogenetic relationship between isolates was done by constructing a Phylogenetic tree using Clustal omega program.

(3) Assessment of polymer degradation potential of bacterial isolates:

Materials:

18 h old isolates grown on Nutrient agar, Sterile Busnell and Haas Broth containing 1g of polyethylene, Gooch crucible, GPC.

Method:

100mL of sterile Busnell and Haas Broth containing 1g of polyethylene was inoculated with 1% of 18 h old isolates grown on Nutrient agar (10^{11} cells/mL). The flasks were incubated at room temperature (28±2^{0}C) under shaking conditions (120 rpm) for 4 weeks.

(a) Growth Response: The growth response of the isolates was monitored turbidimetrically at the end of every week for four weeks.

(b) Weight loss analysis: At the end of 4 weeks of incubation period, the loss in weight of polyethylene was estimated gravimetrically by weight loss analysis using Gooch crucible (Mathur *et al.,* 2011).

The percent weight loss (mass reduction) was computed with the following formula:

$$\% \text{ weight loss} = \frac{(W1-W2)}{W1} \times 100$$

Where, W1 is the pre-incubation weight of LDPE and W2 is the post-incubation weight of LDPE.

(c) Determination of Molecular weight by Gel Permeation Chromatography:

Mn (Number-average molecular weight), Mw (Weight-average molecular weight), Mz (Z-average molecular weight) and PD (Polydispersity) of LDPE samples were determined using Gel Permeation Chromatography.

RESULTS AND DISCUSSION

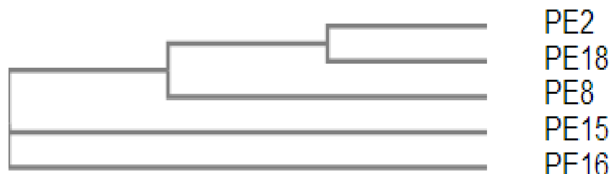
(1) Isolation: A total of 18 Isolates (named PE – 1 to PE – 18) were obtained. Five isolates that showed better growth response to LDPE as compared to other isolates were studied further.

(2) (a) Identification of the isolated bacterial strains by 16s rRNA:

Isolate	16s rDNA sequence	Description
PE-2	GGGGCACTTAATGCGTTAGCTACGGCGCGGAAAACGTGGAATGTCCCCCACACCTAGTGCC CAACGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTC CTCAGCGTCAGTTACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGC GCATTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTCTGCCCGTACCC ACTGCAGAACCGGAGTTGAGCCCCGGTCTTTCACAGCAGACGCGACAAACCGCCTACGAGC TCTTTACGCCCAATAATTCCGGATAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCA CGTAGTTAGCCGGCGCTTCTTCTGCAAGTACCGTCACCCCCAAAGAGGGCTTCTTCCCTAC TGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAGGCTTT CGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCC CAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATTACCCC ACCAACAAGCTGATAGGCCGCGAGTCCATCCAAAACCACAAAAAGCTTTCCACCCCCCACC ATGCGATGAGGAGTCATATCCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGTCAAG GGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTAATCCCCGGCGCAAGCACCGGATC ATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCATCCTGAG	*Arthrobacter* sp. AD1
PE-8	AGGCGGTCGACTTAATGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGTC GACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGC ACCTCAGTGTCAGTATTAGCCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCT ACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCTGCCATACTCTAGCTCGCCAGTT TTGGATGCAGTTCCCAGGTTGAGCCCGGGGCTTTCACATCCAACTTAACGAACCACCTACG CGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTTCGTATTACCGCGGCTGCTG GCACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAAACAGCAAGGTATTAACTTAC TGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCAT GGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCT GGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCC TTGGTGAGCCTTTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTGAGG TCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACG TTGTCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATCATGG AGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTATGCCTGCCGCCAGCGTTCAATCTG A	*Pseudomonas stutzeri strain AT11*

PE-15	TGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTTTACGGC GTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTA TCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTA CACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTCGCCAGTTTTGGATGCAGTTCCCA GGTTGAGCCCGGGGCTTTCACATCCAACTTAACGAACCACCTACGCGCGCTTTACGCCCAG TAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGG TGCTTATTCTGTCGGTAACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAAC TTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTT CGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTC CAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCT CACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCT TTCTCCCCGTAGGACGTATGCGGGATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCA GGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGA	*Pseudomonas taiwanensis strain YLCu18*
PE-16	ACTTAATGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTT TACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTG TCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTC ACCGCTACACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTTGCCAGTTTTGGATGCA GTTCCCAGGTTGAGCCCGGGGCTTTCACATCCAACTTAACAAACCACCTACGCGCGCTTTA CGCCCAGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGT TAGCCGGTGCTTATTCTGTCGGTAACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCC TCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATC AGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTC TCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCC ATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTC CCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCA CTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATCAAGGAGCAAGCTCC CGTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGC	*Pseudomonas putida strain BM38*
PE-18	GGGGCACTTAATGCGTTAGCTACGGCGCGGAAAACGTGGAATGTCCCCCACACCTAGTGCC CAACGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTC CTCAGCGTCAGTTACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGC GCATTTCACCGCTACACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTCTGCCCGTACCC ACTGCAGAACCGGAGTTGAGCCCCGGTCTTTCACAGCAGACGCGACAAACCGCCTACGAGC TCTTTACGCCCAATAATTCCGGATAACGCTTGCGCCCTACGTATTACCGCGGCTGCTGGCA CGTAGTTAGCCGGCGCTTCTTCTGCAAGTACCGTCACCCCCAAAGAGGGCTTCTTCCCTAC TGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAGGCTTT CGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCC CAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATTACCCC ACCAACAAGCTGATAGGCCGCGAGTCCATCCAAAACCACAAAAAGCTTTCCACCCCCCACC ATGCGATGAGGAGTCATATCCGGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGTCAAG GGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTAATCCCCGGCGCAAGCACCGGATC ATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCATCCTGAG	*Arthrobacter sp. AD1*

(2) (b) Construction of Phylogenetic tree:



Key
PE – 2 *Arthrobacter sp. AD1*
PE – 8 *Pseudomonas stutzeri strain AT11*
PE– 15 *Pseudomonas taiwanensis strain YLCu18*
PE – 16 *Pseudomonas putida strain BM38*
PE – 18 *Arthrobacter sp. AD1*

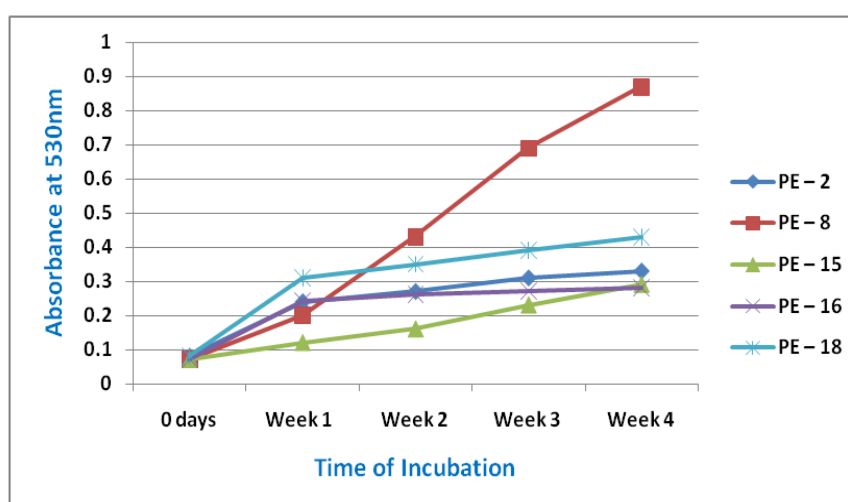
The Phylogenetic tree shows that:

- PE-2 and PE-18 are identical and cluster together.
- PE-2, PE-18 and PE-8 have diverged from a common ancestor.
- PE-15 and PE-16 form a separate clade and cluster together.

(3) Assessment of polymer degradation potential of bacterial isolates:

Table 1: Growth Response of bacterial isolates to polyethylene

Isolate	16s rDNA Identification	Absorbance at 530nm				
		0 days	**Week 1**	**Week 2**	**Week 3**	**Week 4**
PE – 2	*Arthrobacter sp. AD1*	0.08	0.24	0.27	0.31	0.33
PE – 8	*Pseudomonas stutzeri strain AT11*	0.07	0.20	0.43	0.69	0.87
PE – 15	*Pseudomonas taiwanensis strain YLCu18*	0.07	0.12	0.16	0.23	0.29
PE – 16	*Pseudomonas putida strain BM38*	0.07	0.24	0.26	0.27	0.28
PE – 18	*Arthrobacter sp. AD1*	0.08	0.31	0.35	0.39	0.43



- The above graph shows that the isolate PE-8 (*Pseudomonas stutzeri* strain AT11) shows the best growth response to polyethylene.
- The isolate to show growth response second to PE-8 is PE-18 (*Arthrobacter sp. AD1*).

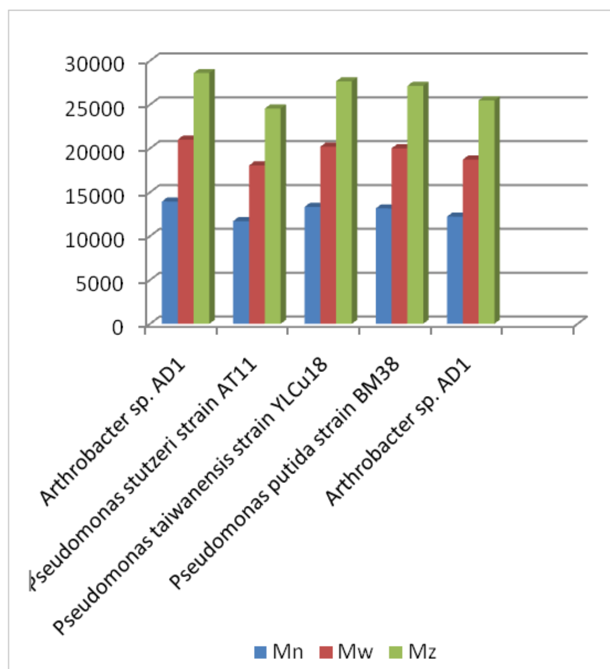
Table 2: Weight loss analysis:

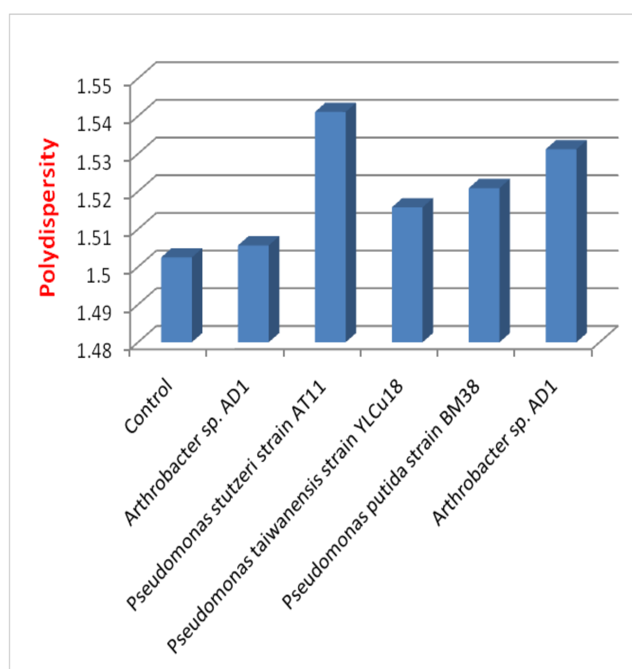
Isolate	16s rDNA Identification	W1 (Pre-incubation weight of Polyethylene in G)	W2 (Post-incubation weight of Polyethylene in G)	% Weight Loss of Polyethylene $\frac{(W1-W2)}{W1} \times 100$
PE – 2	*Arthrobacter sp. AD1*	1.000	0.999	0.100 %
PE – 8	*Pseudomonas stutzeri strain AT11*	1.000	0.980	2.000 %
PE – 15	*Pseudomonas taiwanensis strain YLCu18*	1.000	0.999	0.100 %
PE – 16	*Pseudomonas putida strain BM38*	1.000	0.999	0.100 %
PE – 18	*Arthrobacter sp. AD1*	1.000	0.990	1.000 %

The isolate PE-8 (*Pseudomonas stutzeri* strain AT11) shows maximum degradation of LDPE, that is 2.00% reduction in LDPE weight in 4 weeks of incubation. The second best isolate to is PE-18 (*Arthrobacter sp. AD1*) which shows 1.00% reduction in LDPE weight.

Table 3: Determination of Molecular weight by GPC:

Isolate		Mn	Mw	Mz	PD
Control	-	14334	21537	29209	1.5025
PE – 2	*Arthrobacter sp. AD1*	13952	21008	28589	1.5057
PE – 8	*Pseudomonas stutzeri strain AT11*	11715	18054	24553	1.5411
PE – 15	*Pseudomonas taiwanensis strain YLCu18*	13320	20191	27651	1.5158
PE – 16	*Pseudomonas putida strain BM38*	13154	20007	27133	1.5209
PE – 18	*Arthrobacter sp. AD1*	12227	18722	25477	1.5312





The isolate PE-8 (*Pseudomonas stutzeri* strain AT11) shows maximum decrease in Mw and increase in PD, followed by PE-18 (*Arthrobacter sp. AD1*).

Table 4: Showing five isolates studied for biodegradation of LDPE.

Isolate	% Weight loss of LDPE after 4 weeks of incubation	Mw of LDPE after 4 weeks of incubation
Arthrobacter sp. AD1	0.100 %	21008
Pseudomonas stutzeri strain AT11	2.000 %	18054
Pseudomonas taiwanensis strain YLCu18	0.100 %	20191
Pseudomonas putida strain BM38	0.100 %	20007
Arthrobacter sp. AD1	1.000 %	18722

DISCUSSION AND CONCLUSION:

The compiled results tabulated above show *Pseudomonas stutzeri strain AT11* is the best isolate amongst the five isolates studied for biodegradation of LDPE. This isolate shows maximum growth response to LDPE, maximum % weight loss of LDPE, & maximum reduction in Mw. The results obtained are consistent with the fact that *Pseudomonas stutzeri* has been known to be involved in polymer degradation by production of depolymerases (Sharma, 2004 and Ghosh et al, 2013) and serine hydrolases (Shimao, 2001). The data obtained from the current research is encouraging and eventually will help us device an effective method for Biodegradation of LDPE.

Conflicts of interest: The authors stated that no conflicts of interest.

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