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Polyethylene Degradation by *Pseudomonas aeruginosa* Harboring Catabolic Plasmids

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Abstract: This study determined the plasmid profiles and polyethylene degradation by *Pseudomonas aeruginosa* using standard procedures. Results obtained revealed that 22 (78.6%) of the polyethylene degrading *Pseudomonas aeruginosa* harbors catabolic plasmids. 6 (21.4%) was found without plasmid, 8 (28.6%) had one plasmid, 11(39.3%) had two plasmids while 3(10.7%) had three plasmids each. The sizes of the extracted plasmids ranged from 400bp to 12500bp. Nine different profiles were encountered, 18.2% of the strains (4) presented profile 1, 4 and 9, 13.6% of the strains (3) presented profile 2, 9.1% presented 3 and 4 while 4.5% presented profile 5, 7 and 8. Curing of plasmid at elevated temperature of 45°C resulted in loss of the catabolic plasmids and the organisms subsequently lost the ability to degrade polyethylene. DNA sequence analysis indicated that the plasmid contained six open reading frames, no tRNA genes, three inverted repeats and three direct repeats.

Key words: Polyethylene · Plasmid · *Pseudomonas aeruginosa* · Curing Agents

INTRODUCTION

The increasing rise in polyethylene littering of the environment is a common environmental problem affecting most urban centers in Africa due to its recalcitrant nature to degradation. These wastes are known for causing a number of environmental and health problems to both humans and other animals including over a million deaths to marine animals [1]. Polyethylene, which is a polymer made of long chains of ethylene monomers is growing worldwide at a rate of 12% and about 140 million tons per year. With such a large amount of polyethylene accumulating in the environment, thousands of years are required to efficiently degrade them [2]. Biodegradation is the process in which microorganisms like fungi and bacteria degrade the natural polymers (Lignin, cellulose) and synthetic polymers (Polyethylene, polystyrene) [3].

A bacterium needs the appropriate catabolic genes in order to be a degrader of a compound. Many of the genes involved in the degradation are often located on plasmids [4]. These extrachromosomal elements first came to reckoning with the discovery of their involvement in the spread of bacteria resistance to antibiotics [5]. Plasmids that carry structural genes that code for the degradation of many naturally occurring organic compounds and xenobiotics, are referred to as degradative or catabolic plasmids. The first plasmids that were discovered were predominantly circular plasmids [5, 6], but reports abound today of plasmids with linear configuration and a number of these have been reported as degraders of xenobiotics and other recalcitrant pollutants [7-9].

The genetic flexibility of bacteria has contributed to their survival in altered environments, because of their capacity to acquire and transfer catabolic genes. Bacteria have developed degradative attributes to different classes of organic compounds. The most frequent type of degradation is acquired and transmitted horizontally either through conjugation, transformation or transduction of plasmid. The presence of degradative genes on mobile genetic elements has been fingered as an indication of easy spreading of catabolic abilities among

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bacteria in polluted soil, as a result of conjugative transfer [10]. This is further buttressed by the similarities in the sequence of genes among strains [11]. A plasmid may encode a complete degradative pathway or partial degradative step. Some other plasmids code for enzymes that have specificity for several substrates. This study aimed at determining the role of plasmid in polyethylene degradation by *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Source of *Pseudomonas aeruginosa:* The strains of polyethylene degrading *Pseudomonas aeruginosa* was supplied by Miss Avoseh Zansi Tobi in her previous study. These isolates were further identified and reconfirmed in this study to species level following the guidelines of Analytical profile index (API) (Bio Merieux, Inc).

Plasmid Extraction and Isolation: Plasmid extraction was done using the alkaline lysis method of Takahashi and Nagano [12]. Plasmid DNA bands were detected by electrophoresis on 0.8% horizontal agarose gel pre-stained with ethidium bromide (0.5μg/mL) and visualized under UV light. The sizes of the plasmid DNA bands were determined by extrapolation based on the mobility of Hind III digested DNA co-electrophoresed with the plasmid DNA samples [13-15].

Plasmid Curing: The plasmid curing study was performed for isolate with highest catabolic activity by physical method (Treating cells at 45°C), as described by Foster [5]. The isolate was inoculated in Luria broth (Hi-Media) in duplicate. One flask was incubated at 37°C while the other at elevated temperature (45°C) overnight for plasmid curing. The curing was confirmed by loss of plasmid and catabolism testing using polyethylene as a substrate. The catabolic test was carried out in duplicates. The pre-weighed discs of 1-cm diameter prepared from polyethylene bags was aseptically transferred into conical flask containing 50 ml of nutrient broth, inoculated with plasmid cured *Pseudomonas aeruginosa*. Control was maintained with polyethylene discs in a plasmid containing *Pseudomonas aeruginosa* medium. Each of these treatments was maintained and left in a shaker for one month. The polyethylene discs of both plasmid and plasmid cured strain of *Pseudomonas aeruginosa* were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. Degradation was then measured in terms of weight loss. Plasmid sequencing and in silico analysis

A total of 28 DNA fragments were sequenced following initial assessment of the nucleotide level by BLAST analysis against the *Pseudomonas aeruginosa* plasmid in the NCBI database [16]. Following the alignment of the *Pseudomonas aeruginosa* plasmid DNA sequence using the Geneious software, open reading frames (Orfs) were identified using the NCBI ORF finder [17]. The predicted ORFS were then assessed by BLAST analysis against the NCBI database using the blastp program [18]. Alignments were translated using the Show Translation program; the percentage of adenine and thymine bases (AT) and codon usage patterns were calculated using DNA STATS and Codon Usage available on The Sequence Manipulation Suite [19]. The tRNAs were determined using the tRNAscan-SE program [20]. Inverted repeats and tandem repeats were identified using EMBOSS explorers' inverted program [21]. Insertion sequence elements were identified using the IS Finder database search tool [22].

RESULTS

All the strains grew on Cetrimide agar, were Gram-negative rod shape bacteria, motile by means of single polar flagella, oxidase-positive, do not ferment glucose and were positive to pyocyanin production. Of the 28 strains tested, 22 (78.6%) harbored catabolic plasmid (Table 1) and 6 (21.4%) was found without plasmid, 8 (28.6%) had one plasmid, 11(39.3%) had two plasmid while 3(10.7%) had three plasmids each. The size of the extracted plasmids ranged from 400bp to 12500-bp. Nine different profiles were encountered, 18.2% of the

Table 1: Occurrence of Catabolic Plasmids in Polyethylene degrading *Pseudomonas aeruginosa*

Number of Plasmid	Percentage of occurrence
0	6(21.4)
1	8(28.6)
2	11(39.3)
3	3(10.7)

Table 2: Plasmid profiles of Polyethylene degrading *Pseudomonas aeruginosa*

Plasmid profiles	Frequency of occurrence
1	4(18.2)
2	3(13.6)
3	2(9.1)
4	4(18.2)
5	1(4.5)
6	2(9.1)
7	1(4.5)
8	1(4.55)
9	4(18.2)

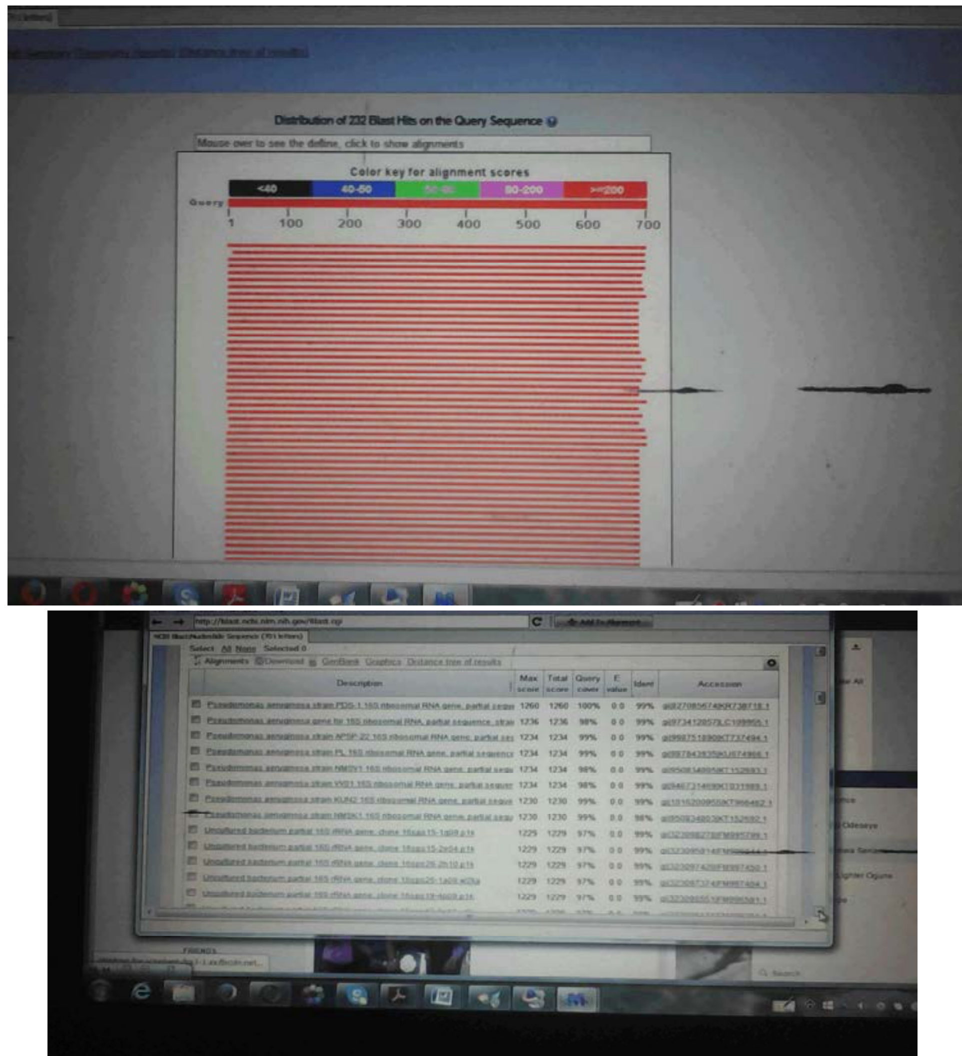

Fig. 1: Graphical summary of *Pseudomonas aeruginosa* blasting

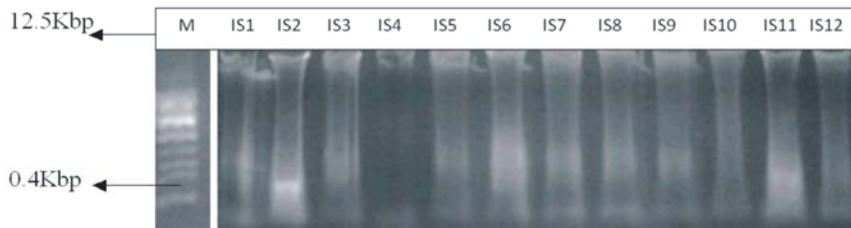

Fig. 1: Agarose gel photograph of plasmid DNA of Polyethylene degrading *Pseudomonas aeruginosa*. Lane 1: Standard plasmid marker; lanes 2 to 13: plasmid DNA of different polyethylene degrading *Pseudomonas aeruginosa* (IS1-1S12), respectively (Not all bands are visible??).

strains (4) presented profile 1, 4 and 9, 13.6% of the strains (3) presented profile 2, 9.1% presented 3 and 4 while 4.5% presented profile 5, 7 and 8 (Table 2). To determine the possible linkage between polyethylene degradation and plasmid DNA, plasmid curing study was conducted for isolate with highest catabolic activity. Curing of plasmid at elevated temperature 45°C resulted in the loss of the plasmids. The cured bacteria eventually lost the ability to degrade polyethylene. DNA sequence analysis indicated that the plasmid contained six open reading frames, no tRNA genes, three inverted repeats and three direct repeats.

DISCUSSION

The use of microorganisms for organic compound degradation has been well documented [12, 13]. In this study, 22 of the 28 polyethylene degrading *Pseudomonas aeruginosa* harbored varying degree of plasmids. This observation is not unexpected as Johnsen *et al.* [4] had earlier reported that bacteria need the appropriate catabolic genes in order to be degrader of a compound because many of the genes involved in the degradation of organic compound are often located on plasmids. They further stressed that these plasmids carry structural genes that code for the degradation of many naturally occurring organic compounds and xenobiotics and thus are referred to as degradative or catabolic plasmids. In another word, Foster [5] stated that a plasmid may encode a complete degradative pathway or partial degradative step. Some other plasmids may even code for enzymes that have specificity for several substrates. The plasmid profiles of *Pseudomonas aeruginosa* have been studied [23] where a high diversity of profiles was observed [24]. Plasmid profiling has been proven useful to differentiate between *Pseudomonas aeruginosa* strains but their discriminatory power has also been questioned [25]. To determine the possible linkage between polyethylene degradation and plasmid DNA, Curing was carried out at elevated temperature (45°C) and this resulted in loss of their degradative attributes. This observation is not surprising because Foster [5] successfully cured 14.3 kb plasmid in *Lactobacillus helveticus* strain ILC 54 at 45°C.

CONCLUSIONS

It can therefore be concluded based on our research findings that the polyethylene degradative marker genes are located on plasmids.

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