P- ISSN 1991-8941 E-ISSN 2706-6703
2020,14 (1):1 - 5

Journal of University of Anbar for Pure Science (JUAPS)

Open Access

Plastic Waste Biodegradation by Local Bacterial Isolates in Ramadi City

Estbrak Yahya Eashur and Mohammed Abbas Jasim*

Department of Biology, College of Education for Women, University of Anbar,Iraq

ARTICLE INFO

Received: 5 / 1 /2020
Accepted: 27 / 2 / 2020
Available online: 6 / 6/ 2020

DOI:10.37652/juaps.2022.172139

Keywords:
Biodegradation,
PET, Plastics,
Pseudomonas fluorescens,
Streptomyces sp., Plastic waste.

ABSTRACT

The current study aims to study biodegradation for plastic waste as one of the ways of treatment of plastic pollution by using isolated bacterial isolates from plastic waste collection and landfill areas in the city of Ramadi and surrounding areas during different periods. Diagnosis of bacterial isolates was performed based on phenotypic and physiological tests as well as testing on the VITEK 2 system, isolates were selected that go back to the type *Pseudomonas* sp. and *Streptomyces* sp. Bacterial isolates have been studied for its ability to breakdown plastic waste used in the manufacturing of drinking bottles and shopping bags by collecting samples of plastic waste and assured they are washed and sterilized perfectly. Another experiment was conducted through a second decomposition (A carbon deficient) medium as well as the second decomposition medium contains some chemicals that are thought to help accelerate the process of decomposition. Results showed that the highest polyethylene breakdown occurred due to isolate P1 (*Pseudomonas fluorescens*) by the degradation rate 8.83% on the first decomposition medium and 30.5% on the second decomposition medium, while the degradation rate to isolated P4 (*Streptomyces*) was 4.83% on the first medium and 19% on the second medium. The genetic study results showed existence of the enzyme PETase gene on *Pseudomonas fluorescens* chromosome in an expected size 119bp and it was absent on plasmid.

1. INTRODUCTION

Evolution in science and technology in the late two decades led to the production of a number of industrial materials as polymers around the world. The polymers are expressions of monomers linked with each other by chemical ligand [1]. In our current age, the plastic includes different kinds of organic and inorganic materials such as carbon, hydrogen, chloride, oxygen, nitrogen, coal and natural gases [2]. Since late 20th century to our days, plastic plays an important role in packaging industries [3]. Plastic usages are widely and contributes to ease our lives, it has many features as light weight, durability, resistance to the rust and low cost, but it difficult to get rid of it and a challenge to solid waste management [4].It has become significantly affecting nature such as the quality of the water and soil [5] Most harmful effects of plastic waste comes from chemicals to the environment, as plastic wastes contribution by 80% of the size of the accumulated waste on the surface of the ocean and coastline [6].

Plastic bags and empty water containers are the largest plastic waste group produced worldwide. Current managements used traditional ways for plastic waste removal by burning, burial and recycling. These ways have some defects and is limited to use because of

*Corresponding author at: Department of Biology, College of Education for Women, University of Anbar, Iraq. Tel.: 00964 7828389947; ORCID: 0000-0003-0857-0676. E-mail address: mohammed.a.jasim@uoanbar.edu.iq

the high costs in addition to the limitation of appropriate PET waste [7], huge amounts of toxic and harmful materials that cause greater pollution of environment, the resurrection of the combustion process accumulates the surface of the earth. Therefore, it is necessary to eliminate this problem or at least reducing it to an acceptable level [8].

Bio-degradation is a biological decomposing of compounds such as polymers normally as a result of applying microorganisms like bacteria, fungi and algae [9].

Decomposition of plastic by microorganisms resulting from the activity of some enzymes that are working on breaking the polymer chain to monomers and oligomers, the plastic material that is entered the enzyme is absorbed by microbial cells to be used in their metabolic processes [10]. Biodegradation for plastic is highlighted by weight loss and change in mechanical and chemical characteristics [11]. Soil microorganisms are responsible for decomposition process. They can use polymer hydrocarbons as a source of carbon. Therefore, biodegradation process is the most suitable alternative method to decrease the problem of plastic wastes that has become serious and threatening the environment.

The current study targeted the evaluation of biodegradation of the slices of PET using selected local bacterial isolates in Ramadi city.

2. MATERIALS AND METHODS

2.1. Samples Preparation

Sixty-four samples of soil and plastic waste were collected from 10 different areas in Ramadi city, the samples were taken using a clean, sterile spatula in 5-15 cm depth, translocated to the laboratory in bags and recorded, some information like sample number, place of collecting and date of combination then are kept in the refrigerator (4°C) for use.

2.2. Bacterial Isolation & Diagnosis

Bacteria were isolated from soil samples by dilution methods, Then the isolates were diagnosed by examined on King B and Gauze agars according to[12].

Isolated bacteria diagnosed depending on classical tests (Oxidase, Catalase, Methyl Red, Indole production, Voges–Proskauer, Citrate utilization, Starch hydrolysis, Urease production, H_2S production, Gelatin liquefaction, Nitrate reduction [13]. These results confirmed later using VITEK2.

Polymer Samples were cut into small slices with 0.03gm for drinking bottles and 0.02 gm. for plastic bags, then transferred to a solution which contains 70 ml of Tween 80, 983 ml of distilled water and 10 ml of bleach and left for 30 minutes, then the slices are washed in distilled water [14].

2.3. Biodegradation of Polymer Samples

Polyethylene slices transferred to 250ml flasks containing 100ml of decomposition medium (Table 1), then inoculated with bacteria, and incubated 28°C for 40 days. Readings taken by removing polymer slices out every 10 days, washed with distilled water and dried back for 24 hours on heat 40-50°C, then weight recorded and re-placed in the decomposition medium, the weight loss was calculated according to the following equation [10]:

$$\text{The missing weight} = \frac{\text{primary weight} - \text{final weight}}{\text{primary weight}} \times \%100$$

Table 1: Decomposing media used in this study [13]

The 1st decomposition medium	
Nutrient agar	8 gm
Bromothymol blue	0.008 gm
D .W .	1000 ml
The 2nd decomposition medium	
NaCl	5 gm
MgSO4	0.2 gm
(NH4)H2PO4	1 gm
K2HPO4	1 gm
Bromothymol blue	0.008 gm
D .W .	1000 ml

2.4. Chromosomal and Plasmid DNA Extraction

Chromosomal DNA extracted from Ps. *fluorescens* using MinigDNA Bacteria Kit while used Plasmid Mini Extraction Kit (Cat. No.: K-3030, K-3030-1) for plasmid extraction both provided by BIONEER company.

2.5.Preparation of Agarose Gel

Agarose gel perpetrated according to [15].

3. RESULTS AND DISCUSSION

The results of the diagnosis revealed that the isolates p1, p3, p5 can be identified as Ps. *fluorescens*, while the isolates p2, p4 belongs to *Streptomyces*. sp., after its comparison with phenotypical and biochemical properties used in bacteria classification [12].

Table 2: Biochemical tests for selected bacterial

No	Tests	Isolates number				
		P1	P2	P3	P4	P5
1	Catalase	+	+	+	+	+
2	Oxidase	+	-	+	-	+
3	Methyl red	-	-	-	-	-
4	Voges-Proskauer	-	-	-	-	-
5	Indole	-	-	-	-	-
6	Citrate utilization	+	-	+	-	+
7	Starch Hydrolysis	-	+	-	+	-
8	Urease	+	+	+	+	+
9	H_2S Production	-	+	-	+	-
10	Nitrate Reduction	-	+	-	+	-
11	Gelatin Liquefaction	+	-	+	-	+

Positive +, Negative -

Table 3: bacterial isolates and their percentages

The bacterial species	Number of isolates	Percentage %
Ps. fluorescens	35	21.34
Streptomyces spp.	23	14.02
Klebsilla spp.	20	12.19
Ps. aeruginosa	18	10.97
Escherichia coli	18	10.97
Acinetobacter spp.	15	9.14
Bacillus spp.	12	7.31
Staphylococcus spp.	10	6.09
Micrococcus spp.	7	4.26
Streptococcus spp.	6	3.65
Total	164	100

Results above confirmed by an advanced technique in diagnosis and determination the bacteria using VITEK2.

The current study results found that Ps. fluorescens isolates isolated from plastic waste contaminated areas has high efficiency in degrading and consumption compounds of poly-ethylene (PET) and use it as a source of carbon and energy compared of *Streptomyces* isolates as the proportion of its degrades respectively 8.83%, 4.83% on the first decomposition as shown in figure (1)

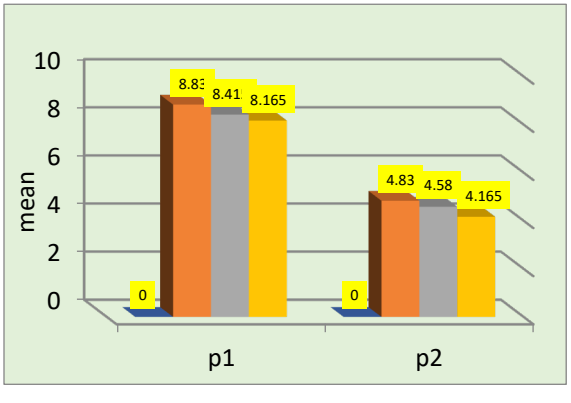

Fig.1: The percentage of polymers decomposition by *Ps. fluorescens* and *Streptomyces*. Sp.

This is due to the strains of *Pseudomonas* which have a group variety of metabolic enzymatic activities against many compounds natural and industrial [16, 17,18].

The results of the current study confirmed by [19] who showed that there are different species of *Pseudomonas*. sp. has ability to break down and analysis of composition (LDPE) and use it as a source of energy and carbon metabolic operations.

As they showed [20] owning the bacterial isolates of the *Pseudomonas* sp. including Ps. *fluorescens* for great capabilities to analyze and dismantle PET more than the rest of the bacterial species.

Pseudomonas sp. and *Streptomyces* sp. isolates showed efficient degradation on the second decomposition medium higher than the first medium as is shown in figure (2), with a degradation rate 30.5% , 19% respectively, This may be as result of containing some nutrients in the first medium which possibly will leads to delay of polymer consumption by bacteria as a source of energy and carbon, in addition having some chemicals in second medium (sodium chloride, sodium hydroxide and nitrates) classified as an enhancing material of degradation of polymers [1, 21].

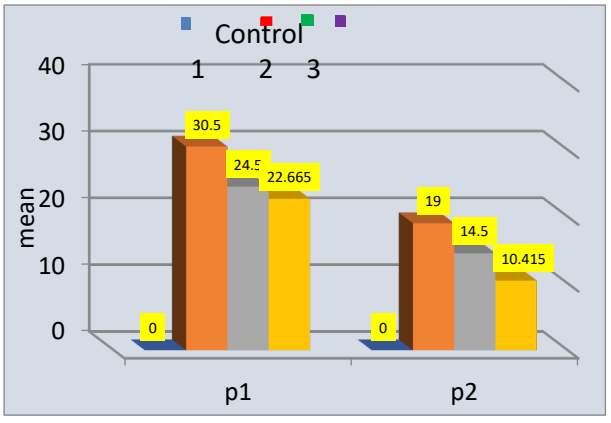

Fig.2: The percentage of polymer decomposition by *Pseudomonas* sp. and *Streptomyces* sp. on second decomposition

Chromosomal DNA and plasmid screened by determined the DNA chromosome from *Ps. fluorescens* using a technique PCR pre mix and The Mini gDNA Bacteria Kit with number GBB100\300 and was deportation electrophoresis in the agarose gel (1.5) and disclosed using the Ethidium bromide and check it out by UV. The result showed that this bacterium on the generous is responsible for an enzyme encryption PETase on the chromosome and was expected size 119bp as in figure (3).

The results of the insulation of DNA plasmid from PS. *fluorescens* showed that these bacteria didn't contain packages of plasmid, although it is productive enzyme PETase. It can be because of encoding of the encrypted by genes on the chromosome, this is consistent with [22] that found 21% from PS. *fluorescens* does not contain plasmids although it has many features analysis of many materials and resistance to many antibiotics.

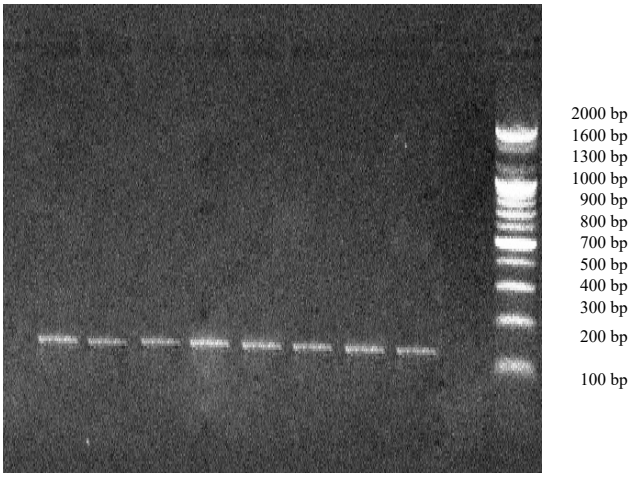

Fig .3: Agarose gel electrophoresis of DNA chromosome from local isolate belong to *Pseudomonas fluorescens*

4. CONCLUSIONS

The results of the current study indicated that biodegradation is environmental safe and inexpensive processing way of plastic waste pollution, the diversity of isolated bacterial isolates of the plastic waste contaminated areas. Also it showed ability to consume PET as a source of energy and carbon. *Pseudomonas fluorescens* have higher degradation rate of PET compared to *Streptomyces* sp. In addition, using chemicals like sodium chloride may increase the speed of the degraded plastics by bacteria.

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التحلل الحيوي للنفايات البلاستيكية بواسطة عزلات بكتيرية محلية في مدينة الرمادي

إستبرق يحيى عاشور و محمد عباس جاسم

قسم علوم الحياة ، كلية التربية للبنات ، جامعة الانبار

E-mail: mohammed.a.jasim@uoanbar.edu.iq

الخلاصة:

استهدفت الدراسة الحالية إلى دراسة التحلل الحيوي للنفايات البلاستيكية كطريقة من طرق معالجة التلوث البلاستيكي عن طريق استخدام عزلات بكتيرية معزولة من مناطق تجميع وطمر النفايات البلاستيكية. عُزلت 164 عزلة بكتيرية من 64 عينة تربة ومخلفات بلاستيكية تم جمعها من مناطق تجمع وطمر النفايات البلاستيكية في مدينة الرمادي والمناطق المحيطة بها وخلال فترات زمنية. أجريت عملية تشخيص العزلات البكتيرية بالاعتماد على الاختبارات المظهرية والفسلجية فضلاً عن اختبارها بنظام VITEK 2 ، وتم انتقاء العزلات التي تعود إلى نوع *Pseudomonas* sp. و*Streptomyces* sp. تم دراسة قابلية العزلات البكتيرية قيد الدراسة على تحليل النفايات البلاستيكية المستخدمة في تصنيع قناني الماء، وأكياس التسوق عن طريق جمع عينات من النفايات البلاستيكية والتأكد من غسلها وتعقيمها ثم أعيدت التجربة على وسط تحلل ثاني يختلف عن وسط التحلل الاول بعدم احتواءه على أي مصدر كاربوني للبكتريا بخلاف قطعة البوليمر فضلاً عن احتواء وسط التحلل الثاني على بعض المواد الكيميائية التي يعتقد أنها تساعد في تسريع عملية التحلل. بينت النتائج أن أعلى تفكك لمادة تيريفثالات متعدد الإثيلين حصل بواسطة العزلة P1 التي تعود لـ *Pseudomonas* بنسبة تحلل بلغت 8.83% وكانت نسبة تحللها على وسط التحلل الثاني 30.5% بينما كانت نسبة تحلل P4 العائدة لـ *Streptomyces* على وسط التحلل الأول 4.83% وعلى وسط التحلل الثاني بلغت نسبة التحلل 19%. من جهة اخرى أظهرت نتائج الكشف الوراثي لصفة انتاج الإنزيم PETase في بكتريا *Pseudomonas fluorescens* وجود الجين المشفر على الكروموسوم البكتيري بحجم 119 زوج قاعدي وعدم وجوده على البلازميد.