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Biodegradation of Plastics by *Pseudomonas* spp. Isolated from Soil Samples of Landfills in Khartoum State, Sudan

Elhadi A. I. Elkhalil and Yousra Abbas

Department of Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum

Abstract: This study was conducted to assess the biodegradation of plastic bags by *Pseudomonas* spp. isolated from soil samples collected from landfills in Khartoum State, Sudan. Soil samples were collected from three different locations (A = Khartoum landfill, B = Khartoum North landfill, C = Omdurman landfill). The Identification and characterizatics of bacteria were done using morphological and biochemical characterizations. The isolated and standard strains were added to the plastic bags in liquid media and then incubated in a rotary shaker for three weeks to allow time for biodegradation. The initial and final dry weights of plastic before and after incubation in the culture medium were compared and the percentage of degradation was calculated. The data were subjected to statistical analysis using completely randomized design (CRD). Mean separations were estimated by Duncan multiple range test (DMRT). This work revealed that the landfill soil is a good source of microbes capable of degrading plastic materials. The isolated strains (*Pseudomonas* spp.) were found to degrade the plastic bags in the range between 76.4 to 84.8 % within three weeks. *Pseudomonas putida* was found to degrade the plastic bags up to 85.2 %. The weight loss in plastic bags' samples is likely due to active enzymes produced by the bacteria.

Key words: Landfills, Plastics bags, Biodegradation, *Pseudomonas* spp.

INTRODUCTION

Use of polythene is increasing day after day and now it can be seen that polythene is being used in almost every activity of life. The polythene demand is expected to increase by 129 % by 2023 (Grover *et al*. 2015). With the increased use of polythene, the pollution level caused

by its random disposal will also be increased, thus affecting almost every kind of environment including terrestrial as well as aquatic biomes. There are various methods for reducing the polythene littering; irrespective of their associated pros and cons (Grover *et al*. 2015). Widespread studies on biodegradation of plastics have been done in order to solve the environmental problems associated with synthetic plastic wastes (Shimo 2001; Shah, *et al*. 2008; Zahra *et al*. 2010). Recent work has included studies of the distribution of synthetic polymer-degrading microorganisms in the environment, the isolation of new microorganisms for biodegradation, the discovery of new degradation enzymes, and the cloning of genes for synthetic polymer-degrading enzymes (Shimao 2001). Although photodegradability had been suggested as a solution for plastic litter (Scott 1990), yet it has limited practical applications because UV light will not penetrate landfills. Among all other methods biological degradation appears to be the most promising solution. Microbes utilize the polythene as a source of carbon and use their enzymatic machinery to solubilize it.

Therefore, it is inferred from the above that there is an urgent need to select and develop efficient microorganisms and with products that may contribute to solving this global issue of plastic disposal (Anonymous l999).This study aims to isolate *Pseudomanas* spp. with the ability to degrade plastics from three soil samples from different dumpimg sites in Khartoum State, Sudan.

MATERIALS AND METHODS

Collection and processing of soil samples

Soil samples were collected from three different dumping sites (landfills) in Khartoum State (A = Khartoum landfills, B = Khartoum North landfills and C = Omdorman landfills). Ten grams of each soil sample were suspended in 100 ml sterile distilled water in a conical flask and placed in shaker at room temperature for 48 hours. They were then diluted with sterile distilled water (6 dilutions for each soil sample; the serial dilution ranged from 10^{-2} to 10^{-6}) and then inoculated onto a selective medium for *Pseudomaonas* spp. that contained 22.8g/l NaCl, 3.97g/l Na_2SO_4, 0.25g/l NH_4CL, 0.41g/l $NaNO_3$, 0.72g/l KCl, 0.19g/l $NaHCO_3$, 0.08g/l KBr, 0.26g/l H_3BO_3 and 0.002g /l NaF in one liter distilled water at 37°C for 24

hours (Inoue, *et al.* 1991). Colonies were then streaked in Petri-dishes containing the selective medium and then the grown colonies were characterized and identified by morphological and biochemical identification methods (Harrigan and McCance, 1966).

Pseudomonas putida (D = *Pseudomonas putida*) which has the ability to degrade the plastic materials was obtained from the Department collections (Department of Agricultural Biotechnology, University of Khartoum,Sudan) as a control and compared with the different isolates from soil samples. Colonies that grew and *Pseudomonas putida* were inoculated into the selective medium containing mineral salt medium as mentioned above for further testing.

Identification and characterization of isolates

The isolates were characterized and identified morphologically and biochemically as described by Harrigan and McCance (1966). These include: Gram staining, motility test, catalase test, oxidase test, oxidative fermentative test, glucose test, sucrose test, lactose test, mannitol test, IMVIC test; (which includes indole test, methyl red test, Voges-Proskauer test, citrate utilization test), triple sugar iron agar test, urease test, nitrate reduction test, gelatin hydrolysis, arginine dihydrolase, lysine decarboxylase and cetrimide resistance tests.

Biodegradation Potential of plastic bags by isolates

Biodegradation of plastic by *Pseudomanas* isolates was tested by adding 0.1 gm of sterilized plastic sample (after autoclaving at 121ºC/ 15 minutes with tween 80) into 100 ml mineral salt medium and then the isolated bacteria from each soil sample as well as *Pseudomonas putida* isolate (control) were inoculated separately. Each isolate was divided into two replicates. They were incubated in rotary shaker (120 rpm) at room temperature for 3 weeks.

Assessment of Biodegradation Percentage of plastic bags

Plastic bags weight loss was determined as described by Coma *et al*. (2006). Plastic bags were removed weekly from the shaker, sterilized with alcohol and the incubated samples were then oven dried at 105ºC for 4 hours. The dried samples were weighed using sensitive balance and were then sterilized with alcohol and transferred to the shaker to continue

incubation. The percentage of degradation of plastic bags for each sample was determined as follows:

$$\text{Bioegradation (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \text{ x } 100\%$$

Statistical analysis

The data obtained were subjected to statistical analysis using statistical Package for Social Science (SPSS version 20). The Duncan multiple range test (DMRT) was used to separate means at $P \leq 0.05$.

RESULTS AND DISCUSSION

Characterization and Identification of Isolates

The results of the various staining, physiological, and biochemical characteristics of the control strain *Pseudomonas putida* and the strains isolated from the different soil samples obtained from the various dumping sites are shown in Table 1. The isolated strains were Gram negative and motile, able to express catalase and oxidase tests. The analysis showed that the isolated bacteria from the soil samples were affiliated to *Pseudomonas* spp. The presence of growth at 28°C suggested that the isolated strains were not *putida* species. The control strain (D) was confirmed as *P. putida* by the absence of both the gelatin hydrolysis test and the growth on cetrimide agar. These results are in agreement with the results of Priyanka and Archana (2011) who isolated *Pseudomonas* spp. from different types of soil.

Table 1. Identification and characterization of *pseudomonas* spp. isolated from soils of different landfills and control strain (*P. putida*)

A=Khartoum landfills, **B** = Khartoum North, **C** = Omdurman landfills, **D** = Standard strain *(pseudomonas putida),* + = Positive test, - = Negative test, K= alkaline, H_2S= Hydrogen sulfite production.

Bacterial Spp / Tests	A1	A2	B1	B2	C1	C2	D
Gram Staining Motility	-	+	-	-	-	-	-
Motility test	Motile	Motile	Motile	Motile	Motile	Motile	Motile
Catalase test	+	+	+	-	-	+	+
Oxidase test	+	+	+	+	+	+	+
Oxidative-fermentative test	Fermentative	oxidative	oxidative	oxidative	Fermentative	Fermentative	Oxidative
Glucose test	+	+	+	+	+	+	-
Sucrose test	+	-	+	+	-	+	-
Mannitol test	+	+	+	+	+	+	-
Lactose test	+	-	+	+	+	+	-
IMViC	+	-	+	-	-	-	+
Triple sugar iron agar test	K/no gas no H_2S	K/no gas no H_2S	K/no gas no H_2S	K/no gas no H_2S	Acid/noH_2O_2	K/no gas no H_2S	K/no gas no H_2S
Urease test	-	+	-	-	-	-	-
Nitrate reduction test	+	+	+	+	-	+	-
Geltin hydrolysis test	+	+	+	+	+	+	-
cetrimide resistance test	+	+	+	+	+	+	+
Growth at 28°C	+	-	+	+	+	+	-
Argnine dihydrolase test	+	-	-	-	+	+	-
Lysine decarbo xlase	+	-	-	-	+	+	-

Biodegradation of plastic bags

Biodegradation of plastic bags was shown in Table 2. The weights of plastic samples were reduced dramatically by all isolates after incubation during the first week. There was no significant increment in the degradation rate with the advancement of the incubation period beyond the first week. The maximum degradation rate was observed after one week of incubation.

Table 2. Weight (g) of plastic samples (Mean ± S.D) incubated with different bacterial isolates for three weeks

Source of Isolates	Incubation time (weeks)			
	Zero time	First	Second	Third
A	$0.10^{a} \pm 0.0$	$0.0279^{b} \pm 0.0007$	$0.0240^{b} \pm 0.0049$	$0.0237^{b} \pm 0.0046$
B	$0.10^{a} \pm 0.0$	$0.0156^{b} \pm 0.0008$	$0.0154^{b} \pm 0.0007$	$0.0152^{b} \pm 0.0008$
C	$0.10^{a} \pm 0.0$	$0.0167^{b} \pm 0.0009$	$0.0166^{b} \pm 0.0011$	$0.0161^{b} \pm 0.0016$
D	$0.10^{a} \pm 0.0$	$0.0150^{b} \pm 0.0014$	$0.0148^{b} \pm 0.0003$	$0.0148^{b} \pm 0.0003$

Means with different letters in the same raw are significantly different at $P \leq 0.05$ according to DMRT

S.D = Standard deviation A = Khartoum landfills

B = Khartoum North landfills C = Omdurman landfills

D = Control strain *(Pseudomonas putida)*

The biodegradation rate of incubated plastic samples as influenced by bacterial isolates and time of incubation is shown in Fig. 1. The rate of biodegradation of plastic samples is significantly different ($P \leq 0.05$) among the bacterial strains. Some of the isolates obtained from soil samples collected from Khartoum North and Omdurman landfills degrade plastic samples in a similar manner to the control strain. The degradation rate was found to be 84.80 % for Khartoum North and 84.0% for Omdurman while the degradation rate for the control strain was found to be 85.20%. The least degradation rate was obtained for the isolates from Khartoum landfills (76.4%). These results are in agreement with the results of Saminathan *et al.* (2014) who reported that *Pseudomonas putida*

is efficient in the biodegradation of plastic materials and the levels of biodegradation for the isolated strains were in the range of 71.7-75.3%. The degradation potential of these microbes may be attributed to the compounds secreted extracellularly by the microbes which may have the ability to break the complex molecular structure of plastics (Priyanka and Archana, 2011).

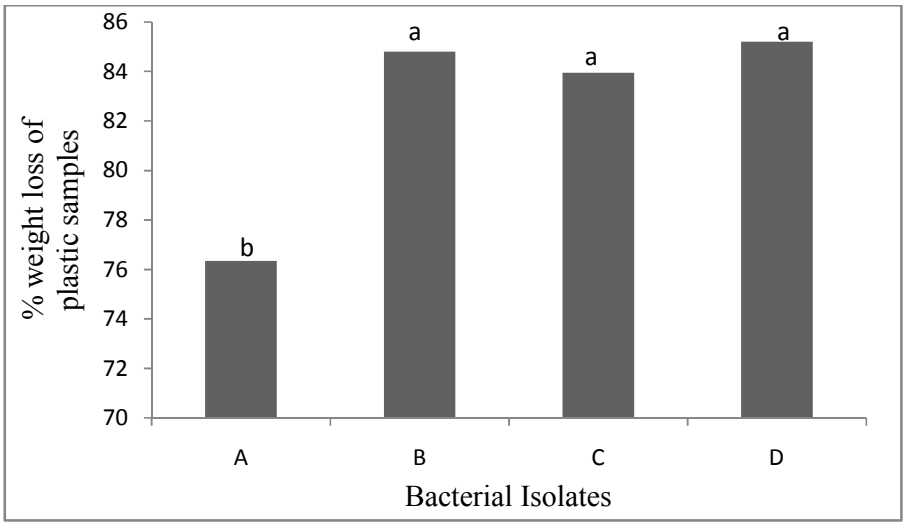

Fig.1 Degradation (%) of plastic samples after incubation with different bacterial isolates

A = Isolate from Khartoum landfills
B = Isolate from Bahary landfills
C = Isolate from Omdurman landfills
D = Control strain *(Pseudomonas putida)*

Letters on top of columns indicate no significant difference of % weight loss according to DMRT at $P \leq 0.05$.

CONCLUSIONS

Based on these results, it may be concluded that (i) The isolated strains used in this study may have involved *Pseudomonas* spp. (ii) With exception of isolated strain A obtained from Khartoum landfills all of the

isolated strains degrade the experimental plastic sample in a similar manner to the control strain (*Pseudomonas putida*). (iii) The magnitude of biodegradation rate (%) ranges between 76.40 % and 84.80 % within three weeks of incubation under appropriate conditions while biodegradation magnitude by the control strain (P*seudomonas putida)* was 85.20 % indicating that there is no significant difference in the efficiency and rate of biodegradation. (vi) Therefore, it may be inferred that *Pseudomonas* spp. can be considered as an efficient microorganism in the biodegradation of plastic wastes.

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التحلل الحيوي للبلاستيك بواسطة أنواع بكتريا *Pseudomonas* المعزولة من ترب مكبات القمامة بولاية الخرطوم، السودان

الهادي علي ابراهيم الخليل و يسرا عباس

قسم النبات و التقانة الحيوية الزراعية، كلية الزراعة، جامعة الخرطوم، شمبات 13314- السودان

E. mail: eaikhalil@yahoo.com

المستخلص: أجريت هذه الدراسة بغرض معرفة التحلل الحيوي لأكياس البلاستيكية بواسطة أنواع بكتريا جنس *Pseudomonas* المعزولة من ترب مكبات القمامة بولاية الخرطوم، السودان. جمعت عينات الترب من ثلاث مواقع (A = مكب قمامة الخرطوم، B = مكب قمامة الخرطوم شمال- بحري، C= مكب قمامة ام درمان). تم تعريف و وصف البكتريا بالطرق المورفولوجية و الكيميائية الحيوية. اضيفت البكتريا المعزولة و البكتريا القياسية للتحكم (*Pseudomonas putida*) إلي الأكياس البلاستيكية في بيئة غذائية سائلة ثم حُضنت في هزاز لمدة ثلاثة اسابيع تحت الظروف المختبرية الملائمة، لمتابعة التحلل الحيوي. تم مقارنة الوزن الإبتدائي والوزن النهائي الجاف لعينات الأكياس البلاستيكية قبل و بعد التحضين و من ثم تم حساب النسبة المئوية للتحلل. خضعت البيانات المتحصل عليها لعملية التحليل الإحصائي بإستخدام التصميم الكامل العشوائي (CRD). حيث تم استخدام اختبار دانكان الاحصائي(DMRT) للفصل بين المتوسطات. أظهرت هذه الدراسة أن ترب مكبات القمامة تعد مصدراً جيداً للميكروبات التي لها المقدرة علي تحليل المواد البلاستيكية حيوياً. أظهرت السلالات المعزولة *Pseudomonas* مقدرتها العالية على تحليل الأكياس البلاستيكية في المدى مابين 76.35 % الى 84.80% في غضون ثلاثة اسابيع بينما أظهرت السلالة القياسية (*Pseudomonas Putida*) مقدرةً عاليةً لتحليل الأكياس البلاستيكية في حدود 85.20 % خلال ثلاثة اسابيع. نقصان وزن الاكياس البلاستيكية ربما يكون بسبب الإنزيمات النشطة التي أفرزتها البكتريا.