International Journal on Emerging Technologies **8**(1): 05-10(2017)

ISSN No. (Print) : 0975-8364
ISSN No. (Online) : 2249-3255

Microbial Degradation of Polythene using Actinomycetes Isolated from Maize Rhizosphere, Forest and Waste Damping sites within Egerton University, Kenya

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(Received 02 January 2017 accepted 25 January 2017)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Polythenes are used in many spheres of human life such as packing of commodities and in construction of green houses and ponds among other uses. As a result when not properly disposed, they contaminate our environment since they are not easily biodegraded. In this study, some polythene papers were buried around growing maize, in the forest and in waste damping site. At the same time, soil samples were separately collected from the three sites, packed in sterilised polythene bags and taken to the laboratory for isolation of actinomycetes. In the laboratory, the soils were dried on the bench for one week. Following this, actinomycetes were isolated using standard methods using starch casein agar. Characterization of the isolates was carried out using cultural, physiological and biochemical means. The polythene were subjected to degradation by the actinomycetes by placing them in conical flasks having starch casein broth and incubating them in shaking conditions at 30^{0}C for one week. Three potential actinomycetes were isolated from Maize soil (EU10, EU15, EU19), forest soil (EU3, EU8, EU13) and damping site soil (EU21, EU25, EU30). The isolated actinomycetes had varying microscopic, physiological and biochemical characteristics. Although there was no significant difference in polythene sheets degradation between maize, forest and damping site soils (F=38, P<0.05), there was significant difference in the polythene sheets degradation among the actinomycetes (F=11.49, P=0.03). Soils from Egerton University main campus has many strains of actinomycetes. The actinomycetes have varying microscopic, physiological and biochemical characteristics. They have a great potential of producing metabolites that degrade polythenes. There is need for massive isolation and screening of the actinomycetes for production of metabolites that are capable of degrading polythene.

Key words: Actinomycetes, degradation, Egerton, Kenya, polythene, rhizosphere.

I. INTRODUCTION

Polythenes are popular as packaging material due to their portability and effectiveness in transportation and construction (Priya *et al*., 2016). In every year, 500 billion to 1 trillion plastic bags are consumed world wide (Roy *et al.,* 2008). The past three decades' have witnessed increased usage of plastic material in food, clothing, shelter, transportation, medical and recreational industries (Hemashenpagam *et al*., 2013). However, polythene is lethal to the environment since it is resistant to biodegradation leading to pollution (Singh *et al*., 2016).

Polythenes are the most commonly found non degradable solid waste that has been recently recognized as a major threat to marine life. Current reports have indicated that polythenes have caused blockage in the intestines of fish birds and marine mammals (Caruso, 2015). Turtles, whales and sea birds mistake polythene wastes for food or get entangled in them resulting in painful injuries and sometimes death (Midhun and Girijasankar, 2015). Marine debris which also include polythene waste is insightly and unwelcoming to beach goers which can result in loss of revenue (Chua, 2013). Also several cases of cow deaths have been reported in Kenya as a result of cows eating these polythene bags that cause stomach and intestinal blockage. This leads to their demise. Other parts of African countries with similar cases have been reported (Ouko *et al*., 2016). Thus over the years biodegradation of plastics has been subject of interest in waste management (Indumathi and Gayanthri, 2016).

Polythene wastes have a major impact on air pollution (Shonozaki *et al.,* 2013). Burning of polythene waste produces unpleasant and chocking smell. Its combustion produces gases and an air borne particulate emission (Narancica *et al*., 2012). The combustion of both low density polythene (LDPE) and high-density polythene (HDPE) has been found to produce volatile organic compounds (VOC) especially olefins, paraffin, aldehyde and dioxins (Poonam *et al*., 2013).

One of the major strategies to facilitate disintegration and subsequent degradation is direct degradation by microorganism using only the polymer as a sole carbon source (Das *et al*., 2013). Previous studies have reported on biodegradation of polythene by bacterial such as actinomycetes. However, studies on biodegradation of polythene by actinomycetes remain sparingly done especially in Kenya (Gnanavel *et al*., 2013).

Actinomycetes are gram-positive organisms that tend to grow slowly as branching elements (Ibrahim *et al*., 2013). In addition, actinomycetes are prokaryotes, produces spores andexhibit powdery growth (Laycock *et al*., 2013). Their similarity to fungi is in the formation of branched aerial mycelium, which profusely sporulates, is clearly noticeable. They are sometimes called higher bacteria hence the name actinobacteria (Sreedevi, 2015). They belong to both mesophilic and thermophilic groups, which broaden the range of habitats inhabited by them (Kumar *et al*., 2013). Actinomycetes are known to produce an extensive range of bioactive compounds including various enzymes having multiple biotechnological applications (Siddiquee *et al*., 2014).

The aim of the current study was to isolateactinomycetes from the rhizosphere of maize, forest soil and damp site soil followed by testing the isolates for the ability of degrading polyrhenes. The biodegradation of polythene when buried in the three soils was also studied.

II. MATERIALS AND METHOD

A. Study area

The study was conducted at Egerton University, main campus Njoro in Kenya. Egerton University is located in Njoro Sub County with coordinates as 0º 23' south, 35º 35' and altitude of 2000m above sea level. Temperatures range between 17-22ºC while the average annual rainfall is 1000mm (Amata *et al*., 2014).

B. Collection and processing of soil samples

Soil samples were collected from 12 maize rhizosphere, forested area and waste-damping site in Egerton University (Njoro Campus). The samples from the three sampling regions were separately mixed to make a composite sample before air-drying them on the benches for one week. This was done to help reduce the population of gram-negative bacteria (Khasabuli and Kibera, 2014). Following this, the samples were separately sieved through 250 µm pore size sieve (United Kingdom). Heat treatment was carried out by placing the samples in 250 ml Erlenmeyer flask and holding it in a water bath at 50ºC for 1 h to prevent growth of other bacterial flora.

C. Preparation of culture media

Isolation of actinomycetes was carried out using starch casein agar (SCA) (soluble starch: 10 g, K_2HPO_4: 2 g,KNO_3: 2 g, casein: 0.3 g, $MgSO_4.7H_2O$: 0.05 g, $CaCO_3$: 0.02 g, $FeSO_4.7H_2O$: 0.01 g, agar: 15 g, filtered sea water: 1000 ml and pH: 7.0±0.1). The medium was dissolved in distilled water as per the manufacturer's instructions before autoclaving at a temperature of 121°C for 15 min. Following this, the medium was supplemented with 25 µg ml^{-1} nystatin to minimize contamination with fungi and 10µg ml^{-1} nalidixic acid to minimize growth of other bacterial species.

D. Isolation of Actinomycetes on culture media

From each of the composite samples, 1 g of soil sample was separately added to a test tube containing 9 ml distilled water and shaken vigorously at room temperature (25 ± 2°C), using an orbital shaker at 200 rpm for 10 min. The test tubes were considered as stock culture for the soil sample. Aseptically, 1 ml aliquot from the stock solution was transferred to a test tube containing 9 ml of sterile physiological saline and were mixed well. From these test tubes, 1 ml of aliquot were again transferred and mixed with another 9 ml of distilled water to make 10^{-2} dilution factor. Similarly, dilutions up to 10^{-6} was made using serial dilution technique for all soil samples (Aouar *et al*., 2012).

After serial dilution, 0.1ml of each sample was separately plated using pour plate technique. The plates were incubated at 28°C, and observed from 5th day onwards for 25 days. After incubation, actinomycete isolates were distinguished from other microbial colonies by characteristics such as tough, leathery colonies which are partially submerged into the agar (Manikkam *et al*., 2015). Colonies with suspected actinomycetes morphology were sub-cultured on yeast extract malt extract agar medium and incubated at 28°C for 5 to 25 days. Pure cultures were inoculated in 10 ml of yeast extract malt extract broth (YMB) and incubated at room temperature (25 ± 2°C) for 24 to 48 h in a rotary shaker (200 rpm). The pure cultures were maintained in slant culture on yeast extract malt extract agar (ISP2) as well as in glycerol broth at 4°C for further studies (Ramendra *et al*., 2016).

E. Laboratory test for polythene degradation

Fresh polyethylene were collected followed by heating at 40ºC in a forced hot air oven for 3 days in addition to cutting in to small strips (100 mg).Polythene degradation process was carried out using standard procedures (Sumathim *et al.,* 2016). The isolated actinomyectes cultures were aseptically placed into separate conical flask containing starch casein broth after which the plastic strips were added.

Following this, incubation at shaking condition at 37ºC for 30 days was carried out. The strips were washed with 70% ethanol, dried over night at 45ºC and then weighed to determine the changes in weight.

F. Field test for polythene degradation

Polythene were weighed in three equal portions then one portion buried 5cm deep in maize rhizosphere, second portion in a forested area and the third portion in wastes damping site. The polythene were left in the soil for 30 days after which they were removed from the soil, washed with 70% ethanol and dried over night at 45^{o}C. Weighing of the polythene was carried out to determine the extent of degradation.

G. Data Analysis

Data analysis was carried out using Microsoft excel spreadsheet and statistical package for social sciences software (SPSS). Comparison of the means after weighing the polythene was carried out using ANOVA.

III. RESULTS

A. Isolation of Actinomycetes on culture media

Based on cultural morphology, three promising actinomycetes were isolated from maize soil and were coded EU10, EU15 and EU 19.

EU 10 and 15 had grey aerial mycelium and cream on the reverse side (Table 1). The isolates had neither soluble pigments nor melanin production. From forest soil, three isolates bearing three different aerial colours EU3 (dull white), EU 8 (Grey) and EU 13 (Green) were isolated. In addition, the isolates had different colours on reverse side viz EU3 (cream), EU 8 (Grey) and EU 13 (Red). EU 13 produced both soluble pigments and melanin. However, soil from the damping site produced light grey (EU21), white (EU25) and dull white (EU30) isolates. The isolates were cream (EU21), white (EU25) and dull white (EU30) on reverse side. None of them produced either soluble pigments or melanin.

B. Microscopy, physiological and biochemical tests

All the isolates were positive for Gram staining and negative for spore and acid fast staining (Table 2). The isolates tolerated 2% NaCl concentration, EU10, EU21 and EU30 could tolerate 5% NaCl concentration while EU15, EU 19, EU 3, EU 8, EU 13 and EU 25 could not. The isolated actinomycetes could not tolerate 10% NaCl concentration. All the isolates tolerated a pH of 5, EU10 and EU8 (7) while non tolerated a pH of 9.

Table 1: Cultural characteristics of the selected isolate.

Sampling region	Code	Aerial mycelium	Colour on reverse side	Soluble pigments	Melanin production
Maize soil	EU10	Grey	Cream	-	-
	EU15	Grey	Cream	-	-
	EU19	White	Brown	-	-
Forested area	EU 3	Dull white	Cream	-	-
	EU 8	Grey	Grey	-	-
	EU 13	Green	Red	+	+
Damping site	EU 21	Light grey	cream	-	-
	EU 25	white	White	-	-
	EU 30	Dull white	Dull white	-	-

+; positive, -; negative

On temperature tolerance, all the isolates tolerated a temperature of 35, EU 15, 3, 8, 21 and 30 (15^{o}C) while none tolerated a temperature of 55 and 5^{o}C. The isolates were positive for Voges Proskaeur, nitrate reduction, catalase, and casein hydrolysis. However, they were negative for indole production, H_2S production and oxidase tests.

C. Polythene degradation by actinomycetes

Among the polythene papers that were buried in the soil, the final weight ranged from 3.4-2.90g in maize soil, forest soil (1.35-1.30) and damping site soil (1.20-1.00) (Table 3). However, the weight of polythene papers subjected to actinomycetes isolated from maize soil varied from (0.89-0.54), forest soil (1.20-0.65) and damping site soil (0.66-0.32). There was no significant difference in polythene paper degradation between maize, forest and damping site soils ($F=38$, $P<0.05$). Conversely, there was significant difference in the polythene papers degradation among the actinomycetes ($F=11.49$, $P=0.03$).

Table 2: Microscopy, physiological and biochemical characteristics.

Test	**Properties of the isolates**								
Cultural characteristic	EU10	EU 15	**EU 19**	**EU3**	**EU8**	**EU13**	EU21	EU25	EU30
Microscopy									
Gram staining	+	+	+	+	+	+	+	+	+
Spore staining	-	-	-	-	-	-	-	-	-
Acid fast staining	-		-		-	-	-	-	-
Physiology									
NaCl(% w/v) tolerance									
2	+	+	+	+	+	+	+	+	+
5	+	-	-	-	-	-	+	-	+
10	-	-	-	-	-	-	-	-	-
pH tolerance									
5	+	+	+	+	+	+	+	+	+
7	+	-	-	-	+	-	-	-	-
9	-		-	-	-	-	-	-	-
Temperature tolerance (^{o}C)									
5	-	-	-	-	-	-	-	-	-
15	-	+	-	+	+	-	+	-	+
35	+	+	+	+	+	+	+	+	+
55	-	-	-	-	-	-	-	-	-
Biochemical tests									
Voges Proskaeur	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-
Casein hydrolysis	+	+	+	+	+	+	+	+	+

+;positive, -;negative

Table 3: Polythene degradation by isolated actinomycetes and actinomycetes in the soil.

S. No	Final weight (g)											
	MS	**FS**	**DS**	**EU10**	**EU15**	**EU19**	**EU3**	**EU8**	**EU13**	**EU21**	**EU25**	**EU30**
1	1.34	3.20	1.00	0.78	0.80	0.70	1.20	0.82	0.92	0.45	0.54	0.61
2	1.32	3.00	1.08	0.77	0.77	0.65	0.99	0.81	0.81	0.51	0.64	0.50
3	1.33	2.90	1.20	0.67	0.67	0.89	0.65	0.73	0.92	0.53	0.32	0.56
4	1.30	3.40	1.00	0.78	0.74	0.54	0.90	0.99	0.98	0.47	0.50	0.54
5	1.35	3.30	1.10	0.79	0.78	0.56	0.80	1.00	0.80	0.50	0.40	0.66

MS; Maize soil, FS; Forest soil, DS; Damping site soil.

IV. DISCUSSION

The actinomycetes isolated from soil obtained from maizerhizosphere, forest and damping site in Egerton University main campus are presented in table 1. The colour of the aerial mycelium and reverse side agrees with an earlier study (Qais *et al*., 2016). According to Jiang *et al.* (2013) the strain of an actinomycetes isolate greatly determine the colour of the colony which could have contributed to the observed results. However, most of the isolates lacked soluble pigment and did not produce melanin, which differs with a previous study carried out in India (Ramendra *et al.,* 2016). These may be attributed to the habitat in which the actinomycetes were growing. Aouar *et al.* (2012) asserts that soluble pigments and melanin production are very important in classifying actinomycetes.

The results of the current study are typical of actinomycetes (Table 2). Gram reaction, and spore staining are diagnostic tools for actinomycetes (Kumar *et al*., 2013). All the isolates grew very well in 2% NaCl concentration while none grew in 10% NaCl which indicates that the isolates are not salt lovers. The results concur with a study carried out by Mangamuri *et al.* (2013). This indicates that the soils from which the actinmyctes were isolated are not alkaline (Qais *et al*., 2016). All the isolates tolerated a pH of 5 with no growth at pH 9 indicating that the actinomycetes do well in acidic medium. This differs with a study carried out by Ambarwati *et al.* (2014) on isolation of actinomycetes from Indonesia. This could have been brought about my overuse of nitrogenous fertilizers in growing of maize coupled with damping of acidic waste materials in forest and the damping site.

On tolerance to temperature, all the isolates did well at 35^{o}C while none grew at 55^{o}C which agrees with a study carried out inRajasthan (Manikkam*et al*., 2015). This suggests that none of the isolates were thermophilic. This could have been caused by the temperature of the soil from which the actinomycetes were isolated. In addition, the results on biochemical test agree with an earlier study (Saker *et al.,* 2015). Similarity in the isolated strains could be a contributing factor (Ravi and Vasantba, 2016).

The weight of the polythene papers after been buried in Maizerhizosphere, forest and the damping site were higher than the values obtained by subjecting the papers to isolated actinomycetes in the laboratory. This agrees with a study carried out in Tamil Nadu(Devadass *et al*., 2016). Although there was no significant difference in polythene degradation when the polythene papers were buried in the three soils, the weight obtained when the papers were buried in damp site were lower than when the polythenes are buried in maizerhizosphere or in the forest which agrees with a study by Sharma *et al.* (2016). The possible reason is that polythene papers are always in the damp site leading to actinomycetes in these areas producing high levels of enzymes for degrading polythene.

When subjected to degradation by isolated actinomycetes, EU21-EU30 gave the lowest weights of polythene papers. This can be attributed to the fact that the actinomycetes were isolated from the dampingsite, which suggest that they have higher enzymes for degrading polythenes (Ravi *et al*., 2016). On the other hand, EU10-EU19 gave lower weights of polythene than EU3-EU13 because the earlier actinomycetes were isolated from maize rhizosphere where they are involved in a symbiotic relationship with maize triggering them to produce unique enzymes (Ariba *et al.,* 2015). This was confirmed by a study carried out by Saminathan *et al.* (2014) in Nepal.

V. CONCLUSIONS AND RECOMMENDATIONS

Soils from Egerton University main campus has many strains of actinomycetes. The actinomycetes have varying microscopic, physiological and biochemical characteristics. They have a great potential of producing metabolites that degrade polythene. There is need for massive isolation and screening of the actinomycetes for production of metabolites that are capable of degrading polythene.

REFERENCE

[1]. Amata, R. L., Otipa, M. J., Waiganjo, M., Wabule, M., Thuranira, E. G., Erbaugh, M. and Miller, S. (2014).Incidence, prevalence and severity of passion fruitfungal diseases in major production regions of Kenya. *Journal of Applied Biosciences*; **20**: 1146–1152.

[2]. Ambarwati, A.Sembiring, L. and Soegihardjo, C. (2012). Antibiotic produced by *Streptomycetes* associated with rhizosphere of purple nut sedge (*Cyperus rotundus* L.) in Surakarta, Indonesia. *African journal of microbiology research*, **6**(1): 52-57.

[3]. Aouar, L. Lerat, S. Ouffroukh, A. Boulahrouf, A. and Beaulieu, C. (2012). Taxonomic identification of rhizospheric actinobacteria isolated from Algerian semi-arid soil exhibiting antagonistic activities against plant fungal pathogens. *Canadian Journal of Plant Pathology,* **34**(2): 165-176.

[4]. Ariba, B. M., Varalakshmi, B. and Umamagheswari, K. (2015). Biodegradation of polythene bag using bacteria isolated from soil. *International journal of current microbiology and applied sciences*, **4**: 674-680.

[5]. Das, M. P., Kumar, S., Rebecca, J. L. and Sharmila, S. (2013). Isolation and identification of LDPE degrading fungi from municipal solid waste. *Journal of chemical and pharmaceutical research*, **5**: 78-81.

[6]. Devadass, B. J., Paulraj, M. G., Ignacimuthu, S., Theoder, P. A. S., Dhabi, N. A. A. (2016). Antimicrobial activity of soil actinomycetes isolated from Western Ghats in Tamil Nadu, India. *Journal of bacteriology and mycology*, **3**(2): 00059-00065.

[7]. Gnanavel, G., Valli, V., Thirumarimurugan, M. and Kannadasan, T. (2012). Degradation of plastics using microorganisms. *International journal of pharmaceutical and chemical sciences*, **1**: 691-694.

[8]. Jiang, Y. Chen, X. Lou, K. and Mao, P. (2013). Cultivable soil actinomycete communities in some areas of western China. *Academia journal of biotechnology*, **1**(1): 1-13.

[9]. Kumari, N. A., Kumari, P. and Murthy, N. S. (2013). A Novel mathematical approach for optimization of plastic degradation. *International journal of engineering trends and Technology*, **4**(8), 3539- 3542.

[10]. Kumar, V., Bisht, G. and Gusain, O. (2013). Terrestrial actinomycetes from diverse locations of Uttarakhnad, India: Isolation and screening for their antibacterial activity. *Iranian journal of microbiology*, **5**(3): 299-308.

[11]. Laycock, B., Halley, P., Pratt, S., Werker, A. andLant, P. (2013). The chemomechanical properties of microbial polyhydroxyalkanoates. *Progress in polymer science,* **38**, 536– 583 (2013).

[12]. Mangamuri, U. K., Muvva, V., Poda, S. and Kamma, S. (2013). Isolation, identification and molecular characterization of rare actinomycetes from mangrove ecosystem of Nizampatnam. *Malays journal of microbiology*, **8**(2): 83-91.

[13]. Manikkam, R., Venugopal, G., Ramasamy, B., Kumar, V. (2015). Effect of critical medium components and culture conditions on antitubercular pigment production from novel *Streptomyces* sp. D25 isolated from Thar desert, Rajasthan. *Journal of applied pharmaceutical sciences,* **5**(6): 15-9.

[14]. Priya, T., Adria, H., Salman, A., M., Haris, S., Usman, S. and Kalim, M. (2016). Role of microbes in degradation of synthetic plastics and manufacture of bioplastics. *Journal of chemical and pharmaceutical research*, **8**(3): 211-216.

[15]. Qais, Y. M. A., Maher, A., Ola, A. and Abdullah, Y. (2016). "New Records of *Streptomyces* and Non *Streptomyces* Actinomycetes Isolated from Soils Surrounding Sana'a High Mountain". *International journal of research in pharmacy and biosciences*, **3**(I3): 19-31.

[16]. Ramendra, S. P., Charu, S., Pragati, S. and Ajay, K. (2016). Isolation and screening of antimicrobial and extracellular pigment producing actinomycetes from Chambal territory of Madhya Pradesh region, India. *Asian journal of pharmaceutical and clinical research*, **9**, (1)157-160.

[17]. Ravi, R. K. and Vasantba, J. J. (2016). Isolation of Actinomycetes: A complete approach. *International journal of current microbiology applied sciences*, **5**(5): 606-618.

[18]. Saker, R., Bouras, N., Meklat, A., Zitouni, A., Schumann, P. and Spröer, C., (2015). *Prauserellaisguenensis* sp. Nov. A halophilic actinomycete isolated from desert soil. *International journal of systematic evolution in microbiology*, **65**: 1598-603.

[19]. Sharma, P., Kalita, M. C. and Thakur, D. (2016). Broad spectrum antimicrobial activity of forest-derived soil Actinomycete, *Nocardia sp.* PB-52. *Frontiers in microbiology*, **7**: 347-350.

[20]. Siddiquee, M., Helali, M., Gafur, A. and Chakraborty, S., (2014). Investigation of an Optimum Method of Biodegradation Process for Jute Polymer Composite. *American journal of engineering research*, **3**(1):200-206.

[21]. Sreedevi, S., (2015). Solid Waste Generation and its Management- A case study. *International research journal of environmental sciences*, **4**(1), 90-93.