

Microbial Degradation of Maize Waste Materials Using Actinomycetes Isolated from Egerton University Soils, Njoro in Kenya

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ABSTRACT

Every harvest season, receives a lot of plant waste material that have a negative effect on the environment. The rate at which bacteria and fungi in the environment decompose these wastes is low. This study aimed to isolate actinomycetes from soils obtained from Egerton University and test the actinomycetes for the ability to decompose plant wastes materials. Soil samples were collected from field 7 in Egerton University, Kenya. Actinomycetes were isolated using starch casein agar medium. Maize stalk and *Grevillea* sp. leaves were collected from field 3 in Egerton University. One hundred grams of the plant materials were mixed with starch casein broth inoculated with the isolated actinomycetes in conical flasks and incubated in an orbital shaker at 28°C for 1 month. The materials were washed using 70% ethanol prior to drying in a hot air oven and determined the weight. The isolate EU 10 presented grey aerial mycelia, EU 13 (Green), EU 15 (Grey) and EU 19 (White). The isolates presented varying morphological, physiological and biochemical characteristics. There was no significant difference in plant wastes degradation between isolates EU 10, EU 13, EU 15 and EU 19 ($F=11.49$, $p=0.07$). Therefore it was concluded that there is need for massive isolation and screening of actinomycetes for production of metabolites that are capable of degrading plant waste materials.

Key words: Actinomycetes, degradation, isolation, microbes, plant wastes

INTRODUCTION

Plant waste materials have been widely used in soil amendments. Each year, enormous agricultural wastes are produced especially at harvest time¹. In the past three decades, maize materials have been increasingly preserved and used as animal feed. When wastes are not properly preserved, then used as substrates by some fungi such as *Aspergillus* sp. that produce aflatoxins which have been shown to be carcinogen². Some structures present in plant wastes are hard to be biodegraded by most microorganisms³. As a result, the wastes pollute the environment thus endangering other life forms⁴. Turtles, whales and sea birds take these wastes leading to biomagnifications of the microorganisms present in the wastes⁵. When deposited into aquatic ecosystems, the wastes become insightful and unwelcoming to tourists which lead to loss of revenue⁶.

Humans and livestock have been adversely affected⁷. When dairy animals feed on the wastes, the metabolites produced by the microorganisms utilizing the wastes get shed via milk. These metabolites some of which are poisonous are taken by humans in milk⁸. This has led to many life threatening diseases. Many livestock have directly died, as a result of eating infected plant wastes⁹.

Thus over the years' biodegradation of environmental wastes has been subject of interest in waste management. Plant wastes have a major impact on air pollution. Burning of plant waste produces unpleasant and choking smell. Agricultural waste combustion produces soot which is suspended in air and taken to other places by wind¹⁰. In addition, some wastes produce Volatile Organic Compounds (VOC) which are life threatening¹¹.

Effective biodegradation of these wastes however is important¹². The materials can be very important biofertilizers when rapidly decomposed¹³. Several previous studies have reported on biodegradation of plant waste materials by bacterial and fungal species for example *Pseudomonas* spp. However, biodegradation by actinomycetes which have been known to play a key role in nutrient turnover in soil remain untouched¹⁴.

Actinomycetes are gram positive organisms that have high G+C in their DNA. In culture, they grow slowly and partially in the growth medium. Recently, actinomycetes have been placed under bacteria. Formally, they had been placed under fungi due to their ability to form branched aerial mycelium, which profusely sporulate¹⁵. Some actinomycetes are mesophilic while others are thermophilic¹⁶. This helps them to colonize wide range of habitats. This study was aimed at isolating actinomycetes from soils of Egerton University and determining their effectiveness on degradation of plant waste materials.

MATERIALS AND METHOD

Study area: The study was conducted at Egerton University, main campus Njoro in Kenya. Egerton University is located in Njorosub country with coordinates as 0° 23' south, 35° 35' and an altitude of 200 m above sea level. Temperature range was between 17-22°C. While the average annual rainfall is 100 mm¹⁷.

Collection and processing of soil samples: Soil samples were collected from 12 sampling points in field 7 in Egerton University. The samples were mixed to make a composite sample before air drying them on the benches for 1 week. This was done to help reduce the population of gram negative

bacteria¹⁸. The sample was sieved through 250 µm pore size sieve. Heat treatment was carried out by placing the samples in a hot air oven at 120°C for 1 h¹⁹.

Preparation of culture media: Isolation of actinomycetes was carried out using Starch Casein Agar (SCA) (Soluble starch; 10 g K₂HPO₄; 2 g KNO₃; 2 g Casein; 0.3 g Mg SO₄.7H₂O; 0.05 g CaCO₃; 0.02 g FeSO₄.7H₂O; 0.01 g agar; 15 g filtered sea water; 100 mL and pH 7±0.1). The medium was dissolved in distilled water as per the manufacturer's instructions before autoclaving at a 121°C for 15 min. The medium was supplemented with 25 µg mL⁻¹ nystatin to minimize contamination with fungi and 10 µg mL⁻¹ nalidixic acid to minimize growth of other bacterial species²⁰.

Isolation of actinomycetes on culture media: From the composite sample, 1 g of the soil sample was added to a test tube containing 9 mL distilled water and shaken vigorously at room temperature (21±2°C), using an orbital shaker at 200 rpm for 10 min. The test tube was considered as stock culture. Aseptically, 1 mL aliquot from the stock solution was transferred to a test tube containing 9 mL of distilled water to make 10⁻² dilution factor. Similarly, dilution upto 10⁻⁶ was made using serial dilution technique. After serial dilution, 0.1 mL of the sample inoculated on starch casein agar using spread plate technique on sterile Petri plates. The plates were incubated at 28°C and observed from 5th day to the 10th day for growth of actinomycetes. After incubation, actinomycetes isolates were distinguished from other microbial colonies by characteristics such as tough, leathery colonies which are partially submerged into the agar²¹. Colonies show typical actinomycetes characteristics were sub-cultured in starch casein agar. The pure cultures were maintained in slant culture on starch casein medium awaiting further studies²².

Test for degradation of plant waste materials: Plant wastes comprising mainly of maize stalk and *Grevillea* spp. leaves were collected from field 3 in Egerton University. The wastes were heat at 150°C in a hot air oven for 1 h. The plant materials were cut into small pieces, weighed to 100 g and placed in conical flasks having sterile starch casein broth. About 2 mL of starter cultures of the isolated actinomycetes were aseptically added into the conical flasks. Incubation at shaking conditions in an orbital shaker at 28°C for 30 days was carried out. The waste materials were washed with 70% ethanol, dried over night at 45°C and then weighed to determine changes in weight²³.

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 agricultural waste was carried out using ANOVA.

RESULTS

Isolation of actinomycetes on culture media: Based on cultural morphology, four actinomycetes were isolated from the soil and coded EU10, EU 13, EU15 and EU19. EU10 and EU15 had grey aerial mycelium and cream on the reverse side (Table 1). The isolates had neither soluble pigments nor melanin production (Fig. 1). EU 13 presented green aerial mycelia while EU 19 had white aerial mycelia. EU 13 had both soluble and melanin pigments while EU 19 lacked both soluble and melanin pigments.

Microscopy, physiological and biochemical characteristics of actinomycetes: All the isolates were positive for gram

staining and negative for spore and acid fast staining (Table 2). The isolates tolerated 2% NaCl concentration, EU 10 tolerated 5% NaCl concentration while EU 13, EU 15, EU 19 did not. All the isolates did not tolerate 10% NaCl concentration. On temperature tolerance, all the isolates tolerated a temperature of 35°C and EU 15 (15°C). All the isolates did not tolerate a temperature of 5 and 55°C. The isolates were positive for vogesproska eur, nitrate reduction, catalase and casein hydrolysis. However, they were negative for indole production, H₂S production and oxidase tests.

Plant wastes degradation by actinomycetes: The weight of plant wastes subjected to actinomycetes degradation varied from EU 10 (70±0.3 to 79±0.2 g), EU 13 (80±0.3 to 83±0.1 g), EU 15 (70±0.1 to 75±0.1 g) and EU 19 (60±0.2 to 65±0.1 g) (Table 3). There was no significant difference in plant wastes degradation between isolates EU 10, EU 13, EU 15 and EU 19 (F=11.49, p=0.07).

Table 1: Cultural characteristics of the selected isolate

Actinomycetes	Aerial mycelium	Colour on reverse side	Soluble pigments	Melanin production
EU10	Grey	Cream	-	-
EU 13	Green	Red	+	+
EU15	Grey	Cream	-	-
EU19	white	Brown	-	-

+: Positive, -: Negative



Fig. 1: Actinomycetes isolates. The isolates were subculture in starch casein agar medium and incubated at 28°C for up to 10 days. The pure isolates were characterised using morphological characteristics

Table 2: Microscopy, physiological and biochemical characteristics

Test	Isolate			
	EU 10	EU 13	EU 15	EU 19
Cultural characteristics				
Microscopy				
Gram staining	+	+	+	+
Spore staining	-	-	-	-
Acid fast staining	-	-	-	-
Physiology				
NaCl (% w/v) tolerance				
2	+	+	+	+
5	+	-	-	-
10	-	-	-	-
pH tolerance				
5	+	+	+	+
7	+	-	-	-
9	-	-	-	-
Temperature tolerance (°C)				
5	-	-	-	-
15	-	-	+	-
35	+	+	+	+
55	-	-	-	-
Biochemical test				
Vogesproska eur	+	+	+	+
Nitrate reduction	+	+	+	+
Indole production	-	-	-	-
H ₂ S production	-	-	-	-
Catalase	+	+	+	+
Oxidase	-	-	-	-
Casein hydrolysis	+	+	+	+

+: Positive, -: Negative

Table 3: Plant wastes degradation by the isolated actinomycetes

S. No.	Final weight (g)			
	EU 10	EU 13	EU 15	EU 19
1	78±0.2	80±0.3	71±0.2	61±0.3
2	77±0.1	81±0.3	70±0.1	65±0.1
3	70±0.3	82±0.2	73±0.2	64±0.3
4	78±0.1	83±0.1	74±0.3	64±0.1
5	79±0.2	80±0.3	75±0.1	60±0.2

Mean ± SEM

DISCUSSION

The actinomycetes isolated from soils of Egerton University main Campus are presented in Table 1. The morphology of the actinomycetes isolates agreed with an earlier study²³. Sundberga *et al.*²⁴ asserted that same strains of actinomycetes present the same morphological characteristics. However, most of the isolates lacked soluble pigment and did not produce melanin. This differed with previous study by Venterino *et al.*²⁵. These may be attributed to variations in the environmental conditions which the isolates inhabited. Woo *et al.*²⁶ demonstrated the significance of pigments production in categorization of actinomycetes. The results on microscopy, physiological and biochemical characteristics of actinomycetes obtained in this study are typical of actinomycetes (Table 2). Gram reaction and spore staining of

actinomycetes are used as diagnostic tools for actinomycetes²⁷.

All the isolates grew in 2% NaCl while none grew in 10% NaCl. This indicated that the isolates could not resist high salinity. The results were similar with those of a previous study carried out by Rani and Kumar²⁸. This suggested that the actinomycetes were not adapted to saline environments²⁹. All the isolates tolerated a pH of 5 with no growth at pH 9 indicating that the actinomycetes do well in acidic medium. This disagreed with a previous study on degradation of lignin by actinomycetes from Indonesia carried out by Abdel *et al.*³⁰. This could have been brought by over use of nitrogenous fertilizers in field 3 in Egerton University. On tolerance to temperature, all the isolates did well at 35°C while none grew at 5 and 55°C which agreed with a study carried out Radma *et al.*³¹. This suggested that none of the isolates were thermophilic. In addition, the results on biochemical tests agreed with an earlier study³². Similarly the isolated strains could be a contributing factor³³.

When the plant wastes were subjected to degradation by the isolated actinomycetes, EU 19 gave the lowest weights. This suggested that EU 19 was more effective in degrading plant materials than the other isolates³⁴. On the other hand, EU 15 gave lower weights than EU 10 and EU 13. This suggested that EU 15 was better in degrading plant materials than EU 10 and EU 13 which differed with a previous study by Lu *et al.*³⁵. According to Pemila³⁶, the mechanisms involved in degradation of wastes materials differ among actinomycetes.

CONCLUSION

It was concluded that soils from Egerton University are rich in culturable actinomycetes. The actinomycetes have varying microscopic, physiological and biochemical characteristics. In addition, the isolates had a great potential of degrading maize wastes which can be used as bio fertilizers.

RECOMMENDATIONS

The actinomycetes should be isolated in large scale. There is also need to test the ability of the isolates to degrade other plant wastes.

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