# Antimicrobial Activity of Streptomycetes Isolated from the Mau Forest Complex in Kenya

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## Abstract

Search for new principles in biocontrol of plant pathogens different from the classical fungicides is of worldwide interest. Members of the genus *Streptomyces* are known to produce about 80% of all known antibiotics in the world. These antibiotics have been applied in medicine, veterinary and in agriculture. Presumptive *Streptomyces* from the Mau Forest Complex were isolated from soils obtained from different sites within the Mau Complex. 270 isolates were screened for antimicrobial activity against selected plant pathogenic bacteria and two fungal plant pathogens i.e. *Erwinia carotovora, Xanthomonas campestris* pv. *campestris, Pseudomonas savastonoi* pv. *phaseolicola, Fusarium moniliforme, Ascochyta rabie;* the reference cultures were: *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus subtilis* ATCC 6633. 14 (0.05%) of the isolates showed antibacterial activity against the test bacteria while 39 (0.14%) isolates had antifungal activity. Ethyl acetate extracts of culture filtrates from the isolates produced significantly different ($P<0.05$) inhibitory effects to some of the tester microorganisms. Five of these isolates had both antifungal and antibacterial activity. There were further characterized using biochemical, microscopy and morphological methods. These methods showed that they were Gram positive and had similarities to members of the genus *Streptomyces*. The study found that soils from Mau Forest complex harbor diverse group of bacteria including *Streptomyces* species that could be exploited for control of plant diseases. There is need to establish their effectiveness in green house and field studies.

**Key words**: Soil *Streptomyces*, plant pathogens, antimicrobial activity, Mau Forest Complex

## Introduction

Plant diseases have resulted in epidemics in many countries in the world, leading to famines and loss of economic returns. Plant diseases are caused by microorganisms such as fungi, bacteria, viruses, viroids, nematodes and parasitic plants. Fungi cause the most of plant diseases. Chemical fungicides have been used commonly to control the fungal plant diseases (Dutky, 2006). However, these plant pathogens have developed resistance to the fungicides due to over-usage and misuse of the chemical pesticides. Environmental pollution by the agrochemicals, pesticide residues in the treated crops and resistance by the pathogens to the pesticides has raised great concern. Therefore, there is a great need to investigate and discover new approaches that are safe and effective. This includes the use of biologically active compounds from plants, bacteria, fungi and yeasts (UlloaOgaz *et al.,* 2015). Metabolites from antagonistic microorganisms have shown promising results in the management of plant diseases and their rapid breakdown renders them environmentally friendly and safe to use (Davila *et al.,* 2016).

The genus *Streptomyces* belong to the order Actinomycetales within the class Actinobacteria. This is a group of filamentous, Gram positive bacteria and have a DNA G+C content of 63-78 mol% (Kampfer, 2006).Members of the genus *Streptomyces* have been exploited economically and biotechnologically. They are the most valuable class of prokaryotes producing bioactive secondary metabolites such as antibiotics, anti-tumor agents, immunosuppressive agents and enzymes (de Lima Procopio *et al.,* 2012; Nagpure *et al.,* 2014; Aftab *et al.,* 2015). The genus *Streptomyces* are especially prolific, producing around 80% of total antibiotic products (de Lima Procopio *et al.,* 2012). More than 60 Streptomycetes antibiotics have found practical application in human and veterinary medicine, agriculture and industry (Madigan and Martinko, 2007).

In the present study, Streptomycetes isolated from soils in the Mau Forest Complex were screened for antimicrobial activity against fungal and bacterial plant pathogens and other bacterial reference cultures. The Mau Forest Complex is the largest closed-canopy forest ecosystem in Kenya and the largest indigenous forest in East Africa, stretching across 400,000hectares (1,544 square miles). The latitude is between 0º 20′ 60″S and the longitude is

between 35º 27′ 32′′E. It lies between 2,000 m and 2,600m above the sea level, on the Western slope of the Mau Escarpment. Soil samples from natural forest, tea farm, floriculture farm and an agricultural farm was used in this study. The Mau Forest Complex provided a good study area in that there are different cropping systems which can be used to compare their effect on distribution and diversity of microorganisms.

## Materials and Methods

### Soil Sampling

Soil samples were collected from different sites within the Mau forest complex. These sites were: natural forests, agricultural areas and tea farm soil. The GPS coordinates of the area are shown in Table 1.

**Table 1: GPS coordinates for the soil sampling areas**

| Sites | Code | GPS coordinates |
|---|---|---|
| Tea farm | TRF | 35º21´27.87E, 0º20´49.67N |
| Natural forest | FOR | 35º21´31.93E, 0º21´49.45N |
| Wheat farm | WHF | 35º89´21.37E, 0º36´21.48N |
| Botanical Garden area | BGR | 35º67´20.50E, 0º20´33.35N |

The soil samples were randomly collected from the mentioned locality using a sterile Soil Auger (20cm in depth, 2.5cm in diameter) (Lee and Hwang, 2002). Soil samples were taken from a depth of 0-10cm and 10-20cm below the soil surface. The samples were air-dried at room temperature for 7-10 days, they were passed through a 0.8 mm mesh sieve and preserved in sterile polyethylene bags at room temperature before isolation of the bacteria.

### Isolation of the Streptomycetes

Ten grams of the samples of air-dried soil was then mixed with sterile distilled water (90 ml). The mixtures was shaken for 1 hour on a rotary shaker and allowed to settle for another 1 hour. Portions of 1ml of soil suspensions (diluted $10^{-1}$) was transferred to 9 ml of sterile water and subsequently diluted to $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$. Inoculates consisted of adding aliquots of $10^{-3}$-$10^{-6}$ soil dilutions to autoclaved starch-casein agar (Kuster and William, 1964; William and Davies, 1965) supplemented with filter sterilized (0.2 µm filters) antibiotic solution containing cycloheximide, and nystatin (0.005% final concentration), polymixin- β sulphate (0.0005% final concentration) and sodium penicillin (0.0001% final concentration) to inhibit non-actinomycete bacteria and fungi (Baltz, 2006). An aliquot of 0.1 ml of diluted samples was

spread plated on the test media. Three replicates were considered for each dilution. After incubation for 4 – 7 days at 28$^{o}$C, the colonies that had developed on the plates were enumerated and expressed as colony forming units (CFU's) per gram of soil.

The isolated colonies of streptomycetes were transferred from the isolation media to a sterile growth media consisting of glucose (10g), yeast extract (1g), potassium nitrate (1g), potassium monohydrogen phosphate (0.1g) and agar (15g) per litre. The plates were incubated at 28°C for six days. Individual colonies of the cultures were isolated and sub-cultured into freshly prepared agar plates in differential media until pure cultures were obtained. The isolates were streaked on differential agar slants and maintained at 4°C and sub-cultured after every three months (Demain and Davies, 1999).

## Cell Morphology and Cultural Characteristics

The cell morphology of the isolates was done by looking at the spore hyphae morphology. Colour determination was also done by cultivating the isolates on yeast-extract malt extract agar, starch casein agar, nutrient agar and inorganic salt agar. Observation was done after 7, 14 and 21 days. The aerial mass colour, presence of soluble pigment and reverse colour was also observed. Microscopic observation was carried out by cover slip method as described in the ISP project. Arrangement of spores on mycelium was observed under high power objective in the light microscope (Shirling and Gottlieb, 1966).

## Biochemical Characteristics

Gram test was done by spreading the broth culture on a glass slide followed by heat drying. The smear was covered with crystal violet for 30-60 seconds and rinsed off with water. The smear was covered with Gram's iodine for 30-60 seconds then decolorized with alcohol and washed with water. Finally the smear was stained with safranin counter stain for 2 minutes. It was rinsed off and left to dry. The slides were viewed at x100 under the light microscope. Citrate utilization was done by inoculating the test isolates in Simon citrate agar media. The isolates were then incubated at 30$^{o}$C for 48hours. Positive test showed growth and blue colour in the media. Nitrate reduction was also tested where the isolates were grown in 5ml nitrate broth and incubated for 96 hours at 30$^{o}$C. 0.1ml of the test reagent (equal volumes of 0.8% sulphilinic acid and 0.5% α-naphthylamin in 5M acetic acid). Positive test showed red colour while negative results were yellow in colour. Catalase test was done by taking a drop of 10% hydrogen peroxide on a clean glass slide. A colony of the isolates was

picked using a wire loop and placed on the drop of hydrogen peroxide production of bubbles showed positive catalase test, while absence of bubbles showed negative results. Carbon utilization was done by growing the isolates in a basal media of peptone water, the source of carbon was 1% of either glucose, lactose, starch, mannitol, L arabinose, fructose and other sugars. Turbidity of the broth was measured using a photometer to see the amount of growth (Bharti and Arora, 2007).

## Screening for Antifungal Activity

Two plant pathogenic fungi were used for screening of antimicrobial effect. These are: *Fusarium moniliformi* which causes blights and ear rots in maize and *Ascochyta rabie* which causes *Ascochyta* blight in chick peas. The fungal test pathogens was grown in PDA (potato dextrose agar) for three days; 8mm disc plug of the fungi was picked using a sterile cork borer and placed in at 8mm hole bored in the middle of a PDA plate. A disc of *Streptomyces* colony on agar was obtained using an 8 mm diameter cork borer and placed in the PDA media containing fungal test pathogens. Antifungal activity around the *Streptomyces* agar discs was evaluated as described in (Table 2) below and the ratings used were modified from those of Lee and Hwang, (2002).

**Table 2: Rating scale for inhibition diameter by *Streptomyces* isolates against fungal pathogens (Modified from Lee and Hwang, 2002)**

| Inhibition diameter | Rating |
|---|---|
| No inhibition | (-) mycelia growth not different from control |
| 5-9 mm (weak inhibition) | (+) partial inhibition of mycelia growth |
| 10-19 mm (moderate inhibition) | (++) almost complete inhibition of mycelia growth |
| >20 mm (strong inhibition) | (+++) complete inhibition, most mycelia will not grow |

Controls were plain agar blocks

## Submerged Cultures and Preparation of Crude Extract

The active isolates were cultivated on Casein Glycerol agar at 28ºC for 7 days. A 0.6cm diameter disk of this agar culture was transferred aseptically to 250ml Erlenmeyer flasks containing 100 ml Casein Glycerol Broth. The inoculated flasks were kept on a rotary shaker at 130 rpm at 28-30ºC for 7 days. Cells were removed by centrifugation of the broth at 5000 rpm for 20minutes. Cell free supernatant was separated using 0.2µm pore size
membrane filter (Millipore) and the filtrate collected as the antibiotic sample (Liu *et al.,* 2008).

### Antimicrobial Activity of the Culture Filtrate

Antimicrobial activity of the culture filtrate was done using the well diffusion method. For estimation of the antifungal activity, a $10^6$ spore suspension of the fungi was prepared and spread plated on PDA plates. The well diffusion method was used to assay for antifungal activity (Acar and Goldstein, 1996).

### Isolation of Antimicrobial Metabolites

Antimicrobial compound was recovered from the filtrate by solvent extraction method following the process described by Westley *et al.,* (1979) and Liu *et al., (*1986). Ethyl acetate was added to the filtrate in the ratio of 1:1(v/v) and shaken vigorously on the rotary shaker at 130 rpm for 1 hour for complete extraction. The ethyl acetate phase that contains antibiotic was separated from the aqueous phase. It was evaporated to dryness in water bath at 80°- 90°C and the residue obtained was weighed. The obtained compound was used to determine antimicrobial activity. 6mm wet paper disks were added to the obtained residue and used to investigate the zone of inhibition around spread plated of the fungal spore suspension on the PDA plates.

## Data Analysis

To determine the distribution and diversity of the isolates, mean values and standard deviation of the isolates was determined. ANOVA was done to test any variations within the mean values. T-test was carried out to determine the differences in the depths of the parameters tested. Screening for antimicrobial activity was done and the data obtained was presented in plates showing the antimicrobial activity.

## Results and Discussion

### Isolation of Streptomycetes for Use in Bioassay

The Mau Forest complex provided a good study area that has a wide range of soil types with different distribution of streptomycetes. The wheat farm soils had higher CFUs $g^{-1}$ of the streptomycetes as compared to the Tea farm soil and natural forest soils (Table 3).

**Table 3: CFUs $g^{-1,}$ pH and moisture content of the isolates**

| | (CFUs) $g^{-1}$ dry Soil 0-10cm | 10-20cm | pH 0-10cm | 10-20cm | Moisture content (%) 0-10cm | 10-20cm |
|---|---|---|---|---|---|---|
| **FOR** | $6.3\times10^5\pm 2.9\times10^{5b}$ | $3.0\times10^5\pm 1.0\times10^{4b}$ | $4.8 \pm 0.2^a$ | $4.7 \pm 0.3^a$ | $10.27 \pm 0.3^a$ | $10.71 \pm 0.2^a$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| **TEA** | $2.2\times10^4$ ± $1.8\times10^{4a}$ | $1.5\times10^4$ ± $1.4\times10^{4a}$ | $4.3\pm 0.1^a$ | $4.4 \pm 0.3^a$ | $11.02 \pm 0.5^a$ | $11.25 \pm 0.2^a$ |
| **WHF** | $9.3\times10^5$ ± $9.9\times10^{4c}$ | $7.6\times10^5$ ± $1.3\times10^{5c}$ | $5.3 \pm 0.1^a$ | $5.3 \pm 0.1^a$ | $4.81 \pm 0.1^b$ | $4.84 \pm 0.1^b$ |
| **BFOR** | $6.1\times10^5$ ± $1.4\times10^{5b}$ | $4.5\times10^5$ ± $1.1\times10^{5b}$ | $6.2 \pm 0.3^b$ | $6.0\pm 0.4^b$ | $10.27 \pm 0.3^a$ | $10.71 \pm 0.2^a$ |
| **B GR** | $3.5\times10^5$ ± $1.9\times10^{5a}$ | $2.8\times10^5$ ± $7.8\times10^{4a}$ | $6.7 \pm 0.1^b$ | $6.2\pm 0.1^b$ | $9.8 \pm 0.2^a$ | $9.94 \pm 0.2^a$ |

Values are means ±SD. The outcomes sharing a common superscript letter in the same column are not significantly different at $P< 0.05$.

The pH of the soil was weakly acidic ranging from pH 4.3 in the Tea farm soil to pH 6.7 in the Botanic garden grass area. Also, soil from the depth of 0-10 cm had slightly higher pH than the lower layer of 10-20cm. There was no significant difference for the pH value of the sites between the two depths. Water content in all sampling sites decreased with the depth. These results agree with the work of Dharumadurai *et al.,* 2009, who also observed that there were more numbers of isolated actinomycetes in agricultural soils as compared to non-agricultural soils. Basilio *et al.,* (2003) isolated at total number of 116 streptomycetes isolates from soils that had a pH of 5.0, the isolates had antimicrobial activity. The wheat farm had low moisture content, yet this is where there was a high $CFUg^{-1}$ of the streptomycetes. From literature, streptomycetes are able to sporulate during dry conditions and hence they are not affected by low moisture content in the soil. GhorbaniNasrabandi *et al.,* 2013 observed that soils from semi-arid area had highest numbers of streptomcyetes as compared to soils from forest, cultivated area and pasture land.

## Antimicrobial Activity of the Isolates

A total of 270 actinomycetes were isolated and screened for antimicrobial activity (Table 4). The initial screening method was used. 14.4% of the isolates had antifungal activity as compared to 5.2% of the isolates that had antibacterial activity. All the isolates with antibacterial activity showed antifungal activity.

**Table 4: Isolates with antimicrobial activity**

| Site | Code | Isolates screened | Antibacterial activity | Antifungal activity |
|---|---|---|---|---|
| Tea soil | TRF | 43 | 3 (7) | 3 (7) |
| TRF Forest | FOR | 33 | 2 (6.1) | 5 (15.2) |
| Wheat farm | WHF | 50 | 3 (6) | 17 (34) |
| Botanic Grass area | BGR | 62 | 2 (3.2) | 3 (4.8) |
| Botanic forest | BFOR | 26 | 4 (15.4) | 11 (42.3) |

| | | | |
|---|---|---|---|
| **TOTAL** | **270** | **14 (5.2)** | **39 (14.4)** |

Values in parentheses are percentages of isolates with antimicrobial activities

Antifungal and antibacterial activity of some of the isolates is shown in Figure 1.

*Figure 1: Antimicrobial activity of the culture filtrate. The first three plates are inhibitory tests with fungi and last three tests demonstrate inhibitory effects on tester bacteria in the primary streak method. Note areas of growth inhibition on tester microorganisms*

The isolates were further investigated by growing them in broth and the culture filtrate used for bioassay. Out of the 39 isolates, eight had broad inhibitory effect on the bacterial pathogens and the fungal pathogens in the culture filtrate (Table 5), (Figure 2).

**Table 5: Antimicrobial activity of the culture filtrate (the values are means and SD)**

| | B.s | S.a | P.a | E.coli | E.car | P.s.p | F.m | A.r |
|---|---|---|---|---|---|---|---|---|
| BFOR3B14 | 16±3.6 | 12.3±2.5 | N/A | N/A | N/A | N/A | 24.7±0.5 | 25±0 |
| BFOR1A1 | N/A | 19.2±2.9 | 9.9±1 | N/A | N/A | N/A | 19.5±1 | 19±0.5 |
| BFOR3B24 | 21±1 | 19±1.4 | N/A | N/A | N/A | N/A | 28±1 | 19±1.5 |
| WHF2B2 | 18.7±1.8 | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| WHF3A15 | 20.3±0.5 | N/A | N/A | N/A | N/A | N/A | 21±1 | 20.7±1.2 |
| BFOR1B22 | 17±1.7 | N/A | N/A | N/A | N/A | N/A | 20.3±0.6 | 18±1 |
| WHF2B16 | 10.3±0.6 | 5.7±1.2 | N/A | N/A | N/A | N/A | 25.7±1.2 | 22±0.5 |
| WHF1A17 | 15.7±1.2 | 7.7±2.5 | N/A | N/A | N/A | N/A | 20±0.6 | 17±1.5 |

B.s (*Bacillus subtilis*), S.a (*Staphylococcus aureus*), P.a (*Pseudomonas aeruginosa*), *E. coli* (*Escherichia coli*), E. car (*Erwinia carotovora*), P.s.p (*Pseudomonas syringae* pv.


*phaseolicola*), F.m (*Fusarium moniliforme*), A.r (*Ascochyta rabie*), N/A means no activity.

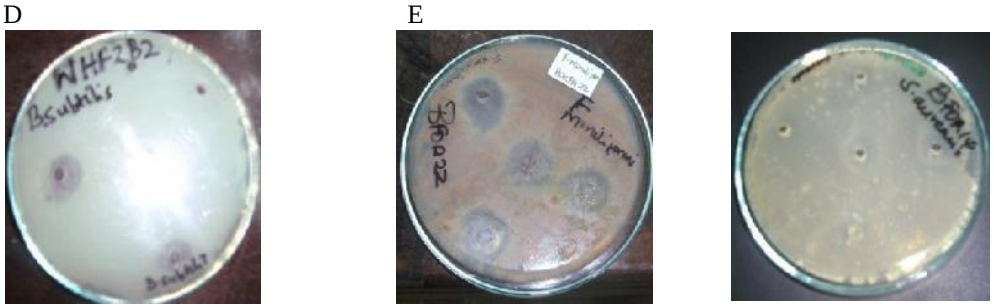



*Figure 2: Antimicrobial activity of the culture filtrate against the tester pathogens*

[A] Antimicrobial of BFOR1 culture filtrate against *F. moniliforme*; [B] BFOR24 against *B. subtilis*; [C] BFOR14 against *B. subtlis*; [D] WHF2B2 against *B. subtilis*, [E] BFOR 22 against *F. moniliforme* and [F] BFOR14 against *S. aureaus.* Zones of inhibition are clearly seen.

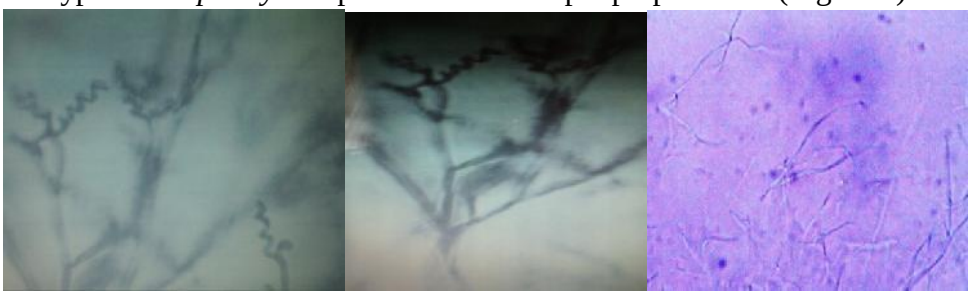
The above results showed that the isolates were more active against Gram positive bacteria and fungi as compared to Gram negative bacteria. Only isolate WHF2B2 did not inhibit the growth of the fungal plant pathogens. All the other isolates showed zones of inhibition against the fungal pathogens. The zones of inhibitions were greater than 15mm and this shows that the isolates have great potential to be used in control of plant fungal pathogens. Hong-Thao *et al.*, (2016) isolated a *Streptomyces* isolate that showed antimicrobial activity against plant pathogenic fungi and *B. subtilis* and *S. aureaus*, the isolate did not have any effect on Gram negative bacteria. Kanini *et al.*, (2013) isolated a Streptomyces spp. that had antifungal activity against *Fusarium oxysporum f. sp. lycopersici*. However, some workers have also isolated *Streptomcyes* sp. that have shown antimicrobial activity against both Gram positive and Gram negative bacteria (Chaudhary *et al.*, 2013; Bizuye *et al.*, 2013).

When the active actinomycetes isolates were grown in submerged culture and the culture filtrate used, the number of bioactive isolates that produced an

inhibition zone on pathogenic microorganisms decreased. Studies have shown that the production of antibiotic compounds is more efficient in solid culture media, compared with submerged media, where activity may decrease or even cease completely. For example research done by Thakur, *et al.,* (2007) showed that out of 65 isolates that showed antibacterial activity in solid medium, 15 failed to do so in liquid medium. Similar results were described by other authors (Salamoni, *et al.,* 2010 and Anibou, *et al*., 2008). Oliveira *et al.,* (2010) suggested this is because the production of antibiotic compounds in liquid media is generally low, and the detection of bioactive compounds requires high concentrations of the compounds.

### Morphological and Cultural Characterization of the Isolates

Gram test showed all isolates were Gram positive and grew well in differential agar media. Growth on the media was abundant with all the isolates showing sporulation. Their colonies were discrete, butyrous, hard to pick and powdery surface. Microscopy was done for the aerial mycelium. The spores ranged from straight, rectus, flexous and verticilli. In some isolates it was possible to see typical *Streptomyces* spores in microscopic preparations (Figure 3).



BFOR3B14 BFOR3B14 WHF3A15

*Figure 3: Examples of spores formed by the isolates with antimicrobial activity*

The color of the isolates on the different growth media is described (Table 6). This is according to the method described in the International Streptomyces Project (ISP). Results from morphological characterization were of qualitative nature and displayed typical characteristics of *Streptomyces* in the Bergey's Manual Sytematic Bacteriology (Whitman *et al.,* 2012). These findings showed that the isolates were presumptive positive for streptomycetes.

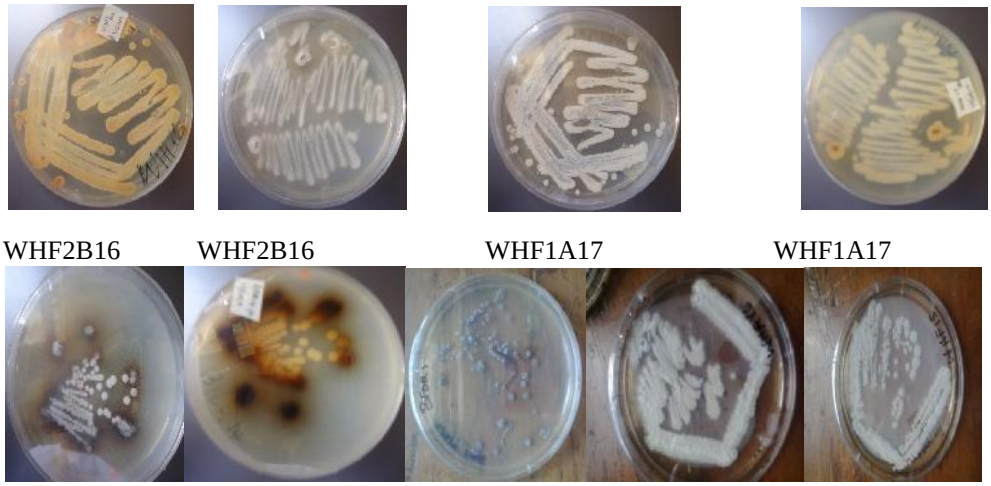
## Results on Biochemical Tests

The biochemical results showed that the isolates were streptomycetes when compared to other members of the class in the Bergey's Manual of

Determinative Bacteriology (Buchanan and Gibbons, 1974). Results from the various biochemical tests conducted are shown in Table 7.

Isolates grew well in differential media. The isolates sporulated in both solid and liquid media. On solid media the isolates were leathery, discrete and tough to pick (Figure 4). All the isolates had abundant growth on basal media supplemented with D- glucose, L-arabinose, starch and casein. WHF1A17 had moderate growth in basal media supplemented with D-xylose the rest of the isolates did not grow in it. The isolates had moderate to doubtful growth on D-mannitol and I-inositol. Isolates BFOR1B22 and WHF1A17 had abundant growth in basal media supplemented with sucrose while the rest had doubtful growth to no growth. Only isolate WHF2B16 liquefied gelatin.

BFOR1A1 and BFOR3B14 had positive catalase test, all the isolates did not grow in Maconkey agar. BFOR1B22 had fair growth on Simon citrate agar.WHF1A17 did not grow on milk agar.



WHF2B16 WHF2B16 WHF1A17 WHF1A17

BFOR3B14 BFOR3B14 BFOR1A1 BFOR1B22 WHF3A15

*Figure 4: Color of isolates on inorganic salt starch agar (ISSA)*

**Table 6: Colour of isolates on different growth media**

| **Media** | **BFOR1A1** | **BFOR1B22** | **BFOR3B14** | **BFOR3B24** | **WHF3A15** | **WHF2B16** | **WHF1A17** |
|---|---|---|---|---|---|---|---|
| **Starch Casein Agar** | | | | | | | |
| Aerial Mycelium | Grey | White | White | Coffee brown | Ivory | Grey | Grey |
| Substrate mycelium | Brown | Brown | Brown | Brown | Pale yellow | Pale yellow | Yellow |
| Soluble pigment | None | None | Dark brown | Dark brown | None | None | Pale yellow |
| **Yeast Malt Extract Agar** | | | | | | | |
| Aerial mycelium | White | Cream | Brown | Brown | Cream | White | White |
| Substrate mycelium | Brown | Pale Brown | Brown | Brown | Cream | Pale yellow | Cream |
| Soluble pigment | None | None | Dark brown | Dark brown | None | None | None |
| **Nutrient Agar** | | | | | | | |
| Aerial Mycelium | Grey | Cream | White | Pale brown | Cream white | Cream | Cream |
| Substrate mycelium | Grey | Pale brown | Brown | Brown | Cream | Pale yellow | Cream |
| Soluble pigment | None | None | Brown | Brown | None | None | None |
| **Inorganic salts starch agar** | | | | | | | |
| Aerial Mycelium | Dull grey | White | White | Brown | White | White | White |
| Substrate mycelium | Brown | Brown | Dark brown | Brown | Pale yellow | Pale yellow | Cream |
| Soluble pigment | None | None | Brown | Dark brown | None | None | Pale yellow |

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**Table 7: Biochemical tests of the isolates with antimicrobial activity**

| | BFOR1B22 | BFOR3B14 | BFOR1A1 | WHF3A15 | WHF2B16 | WHF1A17 |
|---|---|---|---|---|---|---|
| D-Glucose | +++ | +++ | +++ | +++ | +++ | +++ |
| D-Xylose | - | - | - | - | ± | ++ |
| D-Mannitol | ++ | ± | ++ | ± | ± | ++ |
| I-Inositol | ++ | ± | ++ | ± | ± | - |
| L-Arabinose | +++ | ± | +++ | +++ | +++ | +++ |
| Sucrose | +++ | - | ± | ± | ± | +++ |
| L-Rhamnose | ± | ± | ++ | +++ | ++ | + |
| Starch | +++ | +++ | +++ | +++ | +++ | +++ |
| Gelatin | - | - | - | - | + | - |
| Catalase test | - | ++ | ++ | - | - | - |
| Simon citrate | + | - | - | - | - | - |
| Milk Agar | ± | ++ | ++ | ++ | ++ | - |
| Maconkey | - | - | - | - | - | - |
| Casein hydrolysis | ++ | ++ | ++ | ++ | ++ | ++ |

(+++)= Abundant growth, (++) = moderate growth, (+) = fair growth,(±) = Doubtful,(-)=no gowth

## Conclusion

Streptomycetes isolates with antimicrobial activity were isolated from soils in the Mau Forest Complex. The Wheat farm soil had the highest numbers of the isolates in $CFUg^{-1}$, this site also had the lowest moisture content. Streptomycets are spore- producing bacteria and are able to survive in dry soils. Soils from the study area were weakly acidic with a pH ranging from 4.3 in the Tea farm to 6.7 in the Botanic garden soil. The isolates inhibited the growth of Gram positive bacterial and fungal plant pathogens with zones of inhibition that were greater than 20 millimeters. Biochemical, morphological and microscopic studies characterized the isolates as streptomycetes.

## Recommendation

*In-vitro* studies showed that the isolates had antimicrobial activity against plant pathogenic fungi and tester bacteria; we recommend further studies to be done to investigate their application in the field or in a greenhouse set-up. The active metabolites can also be extracted and their structure elucidated

and this can be used to study their application as fungicides and perhaps in making of antibiotics.

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