ISOLATION AND EVALUATION OF ANTIBIOSIS BY *STREPTOMYCES* FROM SOILS OF DIFFERENT LEVELS OF SALINITY

BY

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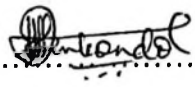
ABSTRACT

This study was undertaken to isolate and screen for antibiosis *Streptomyces* from soils of different levels of salinity. Soil samples for the study were obtained from the Coast region (Chalinze Mzee) and Morogoro region (near Catholic Capuchin Seminary, Kikundi Primary School, Mkata Ranch and SUA farm). Soil samples were analysed for pH, EC, exchangeable bases, % organic carbon, total nitrogen and texture using appropriate methods.Enumeration of *Streptomyces* was done by the plate count method using starch casein agar. Isolation to obtain pure isolates was done on oatmeal agar. Antibiosis testing was carried out on nutrient agar, against *Clavibacter michiganensis* sub sp. Michiganensis, *Xanthomonas phaseoli*, *Xanthomonas phaseolicoli* var fuscoris, *Xanthomonas vasicatoria*, *Xanthomonas oryzae* pv oryzae and *Acidovorax avenae*. Colour of aerial mycelium and morphology of spore chains were determined.*Streptomyces* population ranged from (log_{10}) 2.0 to 4.9 per gram of soil. The study revealed that soil characteristics such as exchangeable bases, extractable phosphorus, organic carbon, and clay content had no significant effect on the number of *Streptomyces*, while total nitrogen and salinity (electrical conductivity) had significant effects.The *Streptomyces* isolates from different levels of salinity were of

various colours such as blue, brown, red, white and their colour intergrades. The spore chains' morphologies of the *Streptomyces* isolates from different soils of different levels of salinity ranged from flexuous, straight, open spirals, open loops and monoverticillate.All the *Streptomyces* isolates were able to grow on media of salt concentration of less than 2%. Some were able to grow on media of salt concentrations between 2% and 8%. No isolate grew on media of 10% salt concentration. The *Streptomyces* showed different degree of antibiosis. Some isolates had strong antibiosis against the test plant pathogenic bacteria, while some had moderate and others had weak antibiosis. Some isolates did not show antibiosis.

DECLARATION

I, Yasin Hassan Senkondo, do declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work and that it has never been submitted for a degree in any other University.

Signature: 

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DEDICATION

This work is dedicated to the family of Hassan Senkondo, my wife, Nasra and my son, Chafweha.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Weight
dS/m	Deci-siemens per meter
EC	Electrical conductivity
ESP	Exchangeable sodium percentage
g	Gram
g/l	Grams per litre
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2PO_4	Sulphuric acid
HCl	Hydrochloric acid
$K_2Cr_2O_7$	Potassium dichromate
Kg	Kilogram
Log_{10}	Logarithm base ten
M	Moles
m	Metre
Mg	Magnesium
Mg/l	Milligram per litre
$MgSO_4$	Magnesium sulphate
mM	MilLimole
Nacl	Sodium chloride
NaHCO3	Sodium bicarbonate
NaOH	Sodium hydroxide
NH^+_4	Ammonium
NH_3	Ammonia
NH_4OAC	Ammonium acetate
nm	Nanometre

P	Probability
ppm	Parts per million
SUA	Sokoine University of Agriculture
USDA	United States Department of Agriculture
UV	Ultra violet
w/w	Weight by weight

CHAPTER ONE

1.0 INTRODUCTION

Soils vary in their types and properties. For example soils are acidic, alkaline, neutral, or saline. However, all are inhabited, to different extents by microorganisms. Alexander (1983) reported that the populations of bacteria in soils ranged from several hundred thousand up to 2 x 10^8 bacteria per gram of soil; fungi ranged from 2 x 10^4 to 1 x 10^6 fungal propagules per gram of soil; and actinomycetes ranged from 1 x 10^5 to 1 x 10^8 propagules per gram of soil in plate count methods depending on soil types from which they were isolated.

The microorganisms perform different functions in the soil. These functions include decomposition of organic residues leading to nutrient cycling. Also, some microorganisms found in soil cause diseases. Others, especially some actinomycete groups, have been proved to produce different kinds of antibiotics (Wellington and Toth, 1994).

Due to their ability to produce antimicrobial compounds, actinomycetes of the genus *Streptomyces* have had great impact in human / livestock

disease control, and have shown potential in plant disease control. The antimicrobial compounds produced have been proved to inhibit development of disease causing organisms such as bacteria, fungi, viruses, and nematodes. For example, *Streptomyces hygrospinosus* var *begingensis* provided protection against tobacco common mosaic (Lin and Piao, 1992) and *Streptomyces pulcher* suppressed development of *Fusarium oxysporum f. sp. Lycopersici* (El Abyad *et al.*, 1993).

Other *Streptomyces* have been proved to produce a number of products for controlling animal diseases such as immunomodulators, enzyme inhibitors, receptor antagonists, and inhibitors of cellular proliferation (Monaghan and Tkacz, 1990). *Streptomyces lydicus* showed strong antagonism against some plant pathogens such as *Pythium ultimum* and *Rhizoctonia solani*, and nematodes such as *Meloidogyne incognita* which cause root galling (Dicklow, *et al.*, 1993). Nemec *et al.* (1996) reported that *Streptomyces griseoviridis* controlled crown rot disease of tomato. *Streptomyces* with such a potential may be also be found in saline environments.

Some studies on the distribution of actinomycetes in soils have been done, but close examination on those studies indicate that information about the

ecology of actinomycetes in various soils is still limited (Williams *et al.*, 1971). However, actinomycetes have now been isolated from alkaline soils (Lacey, 1973). Also, Yuo *et al.* (1982) reported that actinomycetes have been encountered in saline soils. This shows that these environments also harbour these organisms. It is possible that economically important types may be found in these environments also. Therefore, it is important to continue to establish occurrence of *Streptomyces* in our saline soils, to relate the *Streptomyces* populations to extreme soil conditions of salinity, and to identify those types that are adapted to such environments.

The occurrence and antimicrobial potential of *Streptomyces* have been evaluated in different soil types. However, these soil types have included those whose properties were in the normal ranges. Little has so far been done with soils of extreme conditions like those of salinity or alkalinity or on the relationship or the influence of soil characteristics on *Streptomyces* populations.

Currently, the efforts to isolate and screen the *Streptomyces* in Tanzania for purposes of controlling plant diseases have just started. However, the isolation has been done from soils representing a very small area (Morogoro, Iringa, Kibaha and Mbeya regions), and the attention was in

normal soils (Ndonde, 1998). As pointed out above *Streptomyces* have been encountered in salt affected soils, and such soils are used for crop production, for example paddy (Landon, 1991). Little has been reported for saline soils, which represent an extreme soil condition. There is a need not only to evaluate populations of *Streptomyces* under these conditions, but also to explore their potential to produce antimicrobial compounds under these conditions.

Therefore the study reported here was undertaken to explore the *Streptomyces* populations under extreme soil conditions of salinity and to evaluate their potential to produce compounds capable of suppressing plant disease pathogens.

The specific objectives were:

i) To examine the occurrence of *Streptomyces* in soils of different levels of salinity.

ii) To determine the populations of *Streptomyces* in such soils.

iii) To evaluate the tolerance of *Streptomyces* isolates to different levels of salinity.

iv) To evaluate the effects of soil characteristics on *Streptomyces*

populations.

v) To examine the potential of the isolated *Streptomyces* in producing antimicrobial compounds capable of inhibiting selected plant pathogens *in vitro*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Saline or halomorphic environments

2.1.1 General

Soils may be saline, that is enriched with soluble salts in addition to having pH conditions which would otherwise be regarded as being in the normal ranges. These salts would, invariably, exert their own effects on *Streptomyces*. Thus it is felt appropriate to review the concept of salinity here.

Salinity arises due to presence of high concentrations of soluble mineral salts either in water or soils. The major ions of the solutes comprising dissolved mineral salts are the cations Na, Ca, Mg and K and the anions Cl^-, SO_4^{2-}, HCO^-_3, CO^-_3 and NO^-_3. Normally, salinity is expressed as electrical conductivity (EC), the reciprocal of resistance and is reported as deci-Siemens per meter (dS/m) (Brady and Weil, 1996).

Soil salinity can be determined from different extracts of soil, including in saturated soil extracts, in soil solutions extracted by vacuum in the field, using electroconductimetric methods (Tanji *et al.*, 1967).

Salt affected soils are classified as saline, saline - sodic, or sodic. These types of soils occur in many areas in the world such as the United States of America, Canada, Australia, Mexico, Europe, Egypt, South Africa and Tanzania (Abrol, 1988). Traditionally, salt affected soils have been viewed in the light of effects of the salts on plants, especially on crop production (Landon, 1991). Though salinity is detrimental to most plants, some crops are capable of tolerating certain levels of salinity. For example, barley can tolerate salinity levels of up to 8dS/m without much reduction in yields, and can tolerate up to 18 dS/m, but with 50% reduction in yields (Landon, 1991). But the effects of salinity may not be limited only to plants. Soil microorganisms, including *Streptomyces*, occurring in locations which are saline may also be susceptible to detrimental effects of salinity. Thus, populations and activities of *Streptomyces* in saline soils need to be studied to elucidate the effects of salinity on them.

2.1.2 Saline soils

Saline soils contain enough neutral soluble salts to interfere with plants' growth. In saline soils, the electrical conductivity of a saturated extract of the soil solution is more than 4dS/m, exchangeable sodium percentage (ESP) less than 15%, and pH usually less than 8.5 (Brady and Weil, 1996). In Tanzania, these soils are found in some parts of regions such as Morogoro, Sumbawanga, Lindi, and Lower Moshi (De Pauw, 1984). In Morogoro, saline soils have been reported by Kaboni (1996) to occur in the Mafiga - Chamwino lowland area near SUA.

2.1.3 Saline-sodic soils

Saline-sodic soils contain appreciable levels of soluble salts to interfere with plants' growth. They have an EC of more than 4dS/m, ESP of more than 15%. The pH is usually less than 8.5 due to presence of neutral salts. The soils are distributed in locations throughout Tanzania, for example in Dodoma (Bahi Swamps), Iringa, Shinyanga, Mwanza and Kigoma (De Pauw, 1984). Kaboni (1996) encountered saline-sodic soils also in the Mafiga-Chamwino lowland area in the Morogoro municipality.

2.1.4 Sodic soils

Sodic soils have an EC of less than 4dS/m (due to absence of appreciable amounts of soluble salts), ESP of more than 15%, and pH more than 8.5 due to presence of large quantities of exchangeable sodium. Like saline - sodic soils, sodic soils are also distributed in locations throughout Tanzania, including regions such as Dodoma (Bahi Swamps), Iringa, Shinyanga, Mwanza and Kigoma (De Pauw, 1984). These soils have also been encountered in the Mafiga - Chamwino area (Kaboni, 1996).

2.2 Saline Waters

The salinity problem is not limited to soils only, but some waters also may show this property. These are:- saline irrigation and drainage water (deriving their salts, ultimately, from soils), sea water, saline ground waters, brines from natural salt deposits, inland saline lakes and playas, sewage and sludges, and industrial effluents (Richards, 1954).

As already mentioned, water resources such as lakes, rivers and oceans contain salts. Of the total volume of about 1 386 million km^3 of waters, about 96.5% is saline ocean water which is unsuitable for direct human

use (Chassemi *et al.*, 1995). In many countries, the extent of salinity load in different waters is not available. However, the following examples can help to show the extent of salinity in water bodies. In south-east Australia, the river Murray has a salinity level of less than 25mg/l in its lead waters and about 480mg/l downstream at Morgan (Chassemi *et al.*, 1995). In average the river exports 5.5 million tons of salts to the Morgan sea (Chassemi *et al.*, 1995). In the Commonwealth of Independent States, the Aral sea has a salinity level of 2000mg/l and the lake Issyk-Kul has a salinity level of 5800mg/l. In Egypt, the salinity of the Nile river is presently 300mg/l at delta Barrage near Cairo, and the salinity of the Nile river leaving the Nile delta and discharging into Mediterranean sea is about 2 300mg/l (Chassemi *et al.*, 1995). In Tanzania, salt affected water bodies include parts of Lake Tanganyika, and Lakes Manyara, Rukwa, Natron and Eyasi (De Pauw, 1984). The Indian ocean, in common with other oceans, also carries salts (De Pauw, 1984). Kaboni (1996) reported that the Ngerengere river water in the Morogoro municipality had an EC value of 0.3dS/m and, therefore, is categorised as being a low salinity water. Waters originating from Tumbaku and Manzese areas had a level of salinity of 1dS/m and, therefore, are categorised as being medium salinity waters. The Chamwino and Heavy Plant streams had, respectively, 1.6 and 1.5dS/m and, were, therefore, categorised as having

high salinity waters (Kaboni, 1996). Such waters may affect the higher plants as well as microorganisms they may come into contact with, in sediments or soils.

2.3 Halophilic micro-organisms

Some microorganisms may be encountered in halomorphic environments. Such microorganisms may, therefore, be looked upon as being halomorphic or, at least, halotolerant. Brown and Salin (1994) reported that some prokaryotes were halophilic, for example *Halobacterium halobium*. They further commented that for a truly halophilic organism, it would appear likely that a hyposaline environment would present or constitute a stressful environment in much the same fashion as would hypersaline conditions for a nonhalophilic organism. In this respect, metabolic activities of halophilic organisms must have been adapted to the halomorphic conditions prevailing. Gopalakrishnan (1996) reported that rhizobia of *Sesbania bispinosa* could grow at salinity levels ranging from 0 to 3% NaCl. Juniper and Abbott (1993) reported that the population levels of fungi, for example *vesicular arbuscular* mycorrhiza, were highly variable and were influenced partly by soil salinity. Some fungi have been shown to occur naturally in saline environments (Ho,

1987). However, the fungi were not observed where sodium content exceeded 3 131ppm (Juniper and Abott, 1993). In California, the fungi were found in soils of salinity levels of up to 185dS/m (Juniper and Abott, 1993).

Watson and Williams (1973) isolated *Streptomyces* from seawater that were halomorphic. When they tested for salinity tolerance they found that some were able to grow on media of salt concentration of 1.64M NaCl (a salt concentration four times that of sea water) and they concluded that the *Streptomyces* strains were halomorphic.

2.4 Effects of salinity on populations of micro-organisms

In normal soils, *Streptomyces* populations ranged from (log_{10}) of 4.6 to 5.8 per gram of soil (Ndonde, 1998; Philip, 1998; Ikerra, 1986; Keya, 1998). In saline soils, *Streptomyces* populations of up to (log_{10}) 2.24 per gram of soil were encountered (Goodfellow and Williams, 1983). This shows that saline environments are detrimental to most microorganisms. Weyland (1969) reported the population of actinomycetes in the Atlantic Ocean and the North sea. He reported that the populations (expressed as log_{10}) were 1.36 to 3.34 actinomycetes per cm^3 in the North sea. In the

Atlantic Ocean, actinomycetes (as $\log_{10}$) ranged from 1.36 at 175 miles offshore and a depth of 3 362m to 2.13 at 40 miles offshore and a depth of 299m. In this study, 1 348 strains of actinomycetes were isolated from marine sediments. The isolates included *Norcardia, Micromonospora, Microbiospora* and *Streptomyces*. Weyland (1969) observed that a high percentage of sediment samples collected contained actinomycetes, detectable even from millilitre-size samples. He concluded that these microorganisms in the sea seemed to be neither random individuals nor temporary survivors of terrestrial run-off, but must be part of the marine ecosystems.

Watson and Williams (1973) reported actinomycete populations (as $\log_{10}$) of 2 per gram of soil in a coastal sand belt near sea water, 1.78 to 4.6 per gram of soil in beach soils/sands, and 1.69 to 5.9 in the roots of sand-dune plants. In the sea water, *Streptomyces* made 50% of the population and in beach soils they made up to 68% while around the roots of dune plants they made up to 80% of the total population (Watson and Williams, 1973). In seawater the chloride content (w/w) (as an expression of salinity) was 0.34, while in beach it was 0.11 - 0.2 and in the dunes it was 0.003 - 0.004 (Watson and Williams, 1973). This shows that salinity affected the populations of *Streptomyces*, the populations being lowest in

seawater where salinity was highest. This effect was, probably, due to interference of some physiological processes of *Streptomyces* by salts.

Watson and Williams (1973) tested *Streptomyces* strains for salinity tolerance. Of the ten strains tested which were derived from seawater, 100% of strains were able to grow on media whose salt concentration was simulated to that of sea water (0.41M NaCl). Some 75% of the strains were able to grow at 0.82M NaCl, 62% grew at 1.23M and 25% were able to grow at 1.64M. For soils from a beach which had lower salinity as compared to that of the sea, 100% of the 12 isolates tested were able to grow on media simulated for salt concentrations of sea water, and none grew on media of 0.82M NaCl or above (Watson and Williams, 1973). For soils from the dune which were the most hypotonic of all, 93% of the 31 strains tested grew on media of 0.41M salt concentration, 73% grew on media of 0.82M salt concentration and 33% grew on media of 1.23M. None grew at 1.64M (Watson and Williams, 1973). This shows that high salinity levels affected *Streptomyces* populations. Most of the strains which grew at 1.64M were those *Streptomyces* isolated from seawater. This shows that some *Streptomyces* from seawater tolerated high levels of salinity and, therefore, may have been well adapted to that salty environment.

2.5 Effects of salinity on metabolic activities of microorganisms

Some physiological activities of some microorganisms are hampered by the presence of salts. Shlomo *et al.* (1992), for example, he reported that presence of salts in soil due to irrigation with salt water reduced carbon mineralization by 74% as compared to soils that were irrigated with normal water. In the same study Shlomo *et al.* (1992) reported that nitrogen mineralization was reduced by 50%. Band *et al.* (1992) reported that in saline environments polysaccharides are converted to soluble molecules which were used for osmoregulation rather than being used for the normal physiological processes. Pollenko *et al.* (1981) reported that osmotic stress in saline environments caused proliferation of microbial numbers, hence increasing microbial biomass. A salt medium with osmotic potential of up to 1 Mega pascal (MPa) increased microbial biomass (Killham *et al.*, 1990). Killham *et al.* (1990) attributed this to adaptation of the microorganisms to high osmotic pressure levels. Brown and Salin (1994) reported that *Halobacterium* cells subjected to a low salt environment induced a mesophilic catalase (enzyme) with a high specific activity and low salt requirement. They concluded that reduction of the salt content in the growth medium of halophilic organisms may have a physiological relevance, that hyposaline conditions resulted in a critical

need for osmoregulation.

For halophilic microorganisms, imposed hyposaline environments may upset some physiological processes. Brown and Salin (1994) reported that when halophiles experienced relatively hyposaline conditions, there was a need for osmoregulation. Oxygen solubilities would be greater in these hyposaline environments, thereby increasing the potential for production of active oxy-intermediates during metabolism. *Halobacterium halobium* grown in media containing abnormally low salt concentrations induced enzymes associated with the detoxification of active oxygen intermediates (Brown and Salin, 1994). In addition, elevated respiration associated with hyposaline conditions correlated with superoxide dismutase and peroxidase induction, as well as with transiently rapid cell growth rates (Brown and Salin, 1994). *Streptomyces,* being prokaryotes, may too develop similar mechanisms to combat osmotic problems if placed in conditions of different levels of salinity.

Harris (1981) has explained the mechanism of adaptation. He reported that under osmotic stress, most prokaryotes tended to accumulate amino acids and quaternary ammonium compounds as compatible solutes. *Streptomyces,* being prokaryotes also, may have a similar behaviour. In

explaining some mechanisms of adaptation to salinity by micro-organisms, Demple (1991) reported that there are a group of genes (such as oxyR, soxRS, soxQ, katF, arcAB and fur regulon) that have been linked to environmental stress and to the control of enzyme systems that scavenge active oxygen intermediates such as superoxide dismutase, catalase, and peroxidase. Such mechanisms may also operate in *Streptomyces.*

2.6 Effects of salinity on antibiotic production by micro-organisms

The influences of high salinity levels on microbial metabolic activities have been reviewed in section 2.5. Antibiotic production, including that by *Streptomyces*, may also be affected in some way and/or degree by presence of salts in the growth environment. Glazebrook *et al.* (1992) reported that the production of the antibiotic 5-hydroxy-4-oxonorvaline by *Streptomyces akioshiensis* was enhanced by the presence of high concentrations of magnesium salts, and phosphates. They commented that the requirement of Mg salts and phosphate led to the formation of a poorly soluble magnesium-ammonium-phosphate complex in the culture medium which might be an important factor in the synthesis of the antibiotic. The beneficial effects of magnesium and phosphate salts could

also be attributed to their ability to trap the ammonia released during the rapid metabolism of easily assimilated amino acids. Glazebrook *et al.* (1992) further reported that at 108mM phosphate concentration and 10, 5, and 2g/l $MgSO_4.7H_2O$ *Streptomyces* yielded, respectively, 10.5, 10.1 and 8.3 (mM) of the antibiotic. Omura *et al.* (1980) reported that sequestering of free ammonium from the broth by magnesium phosphate stimulated production of macrolide antibiotics that were subject to nitrogen catabolite regulation. Therefore, the antibiotic production was enhanced by increased levels of magnesium.

The effect of ammonia on antibiotic formation has been explained by Lounes *et al.* (1995). It was reported that production of spiramycin antibiotic produced by *Streptomyces ambofacience* was reduced by presence of large quantities of ammonia. The decrease in spiramycin production was attributed to the diversion of spiramycin precursor (such as isobutyrate) pool to the tricarboxylic acid cycle for cellular constituent formation, so that these precursors would not be available for spiramycin biosynthesis. Lounes *et al.* (1995) further reported that ammonium acted by decreasing the availability of short chain fatty acids within the cell, as a consequence of slowing down the catabolism of some of amino acids which are suppliers of these precursors. Ammonium also suppresses

synthesis of acetate kinase and acylphosphotransferase, the enzymes responsible for activation of acetate and propionate, respectively.

He *et al.* (1994) reported that production of antibiotic armentomycin by *Streptomyces armentosus* was enhanced by 10mM chloride concentration. The antibiotic production was reduced at higher chloride concentrations such as 20, 40 and 80mM.

2.7 Effects of salinity on stability of antibiotics

Jefferys (1952) and Pramer and Starkey (1953) reported that streptomycin underwent chemical and microbial decomposition, and could also undergo oxidation, reduction, hydrolysis and/or deamination processes induced by metallic ions (as would be found in saline soils), water, temperature and soil reaction. Thus, stability of antibiotics decreased in high salt conditions. Lee *et al.* (1997) reported that the antibiotic clavanin exerted antimicrobial activity at 0.1 to 0.3M NaCl but it could not exert antimicrobial activities above 0.3M NaCl concentration. This shows that the salts probably denatured the antibiotic. Lai *et al.* (1997) reported that degradation of chloramphenical decreased when salt concentration was increased from 24 to 36 parts per trillion. The degradation of

oxytetracycline was not affected by the increased salt concentration. This shows that antibiotics may or may not be affected by salt concentrations depending on the nature of the antibiotic.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Locations of areas sampled

Soil samples for the study were taken from Coast and Morogoro regions in locations known to contain saline soils. The location within the Coast region was Chalinze Mzee along the Chalinze - Segera - road 3.5 kilometres from Chalinze. Those in Morogoro region were near Catholic Capuchin Seminary along the Morogoro - Dar es Salaam road, about four kilometres from Morogoro town. Other Morogoro locations were near Kikundi Primary School along the Sokoine University road, near Mkata ranch and some 400m west of the SUA main gate along the Morogoro - Mzinga road.

3.2 Soil sampling and routine analysis

Soil samples were collected from the study areas to the depth of 10cm. A composite soil sample of about one kg was taken, placed in a plastic bag and transported to the laboratory where it was subdivided into two equal

portions. One 500g portion was stored in a cool dry room and the other was air - dried, ground and passed through a 2mm sieve for laboratory analysis. The parameters analysed were soil pH, organic carbon, total nitrogen, extractable phosphorus, electrical conductivity, exchangeable bases and particle size distribution.

3.2.1 Soil pH determination

Soil pH was determined in water. A 10g sample was mixed with 25ml of water and shaken for 30 minutes. The pH was determined using the glass electrode.

3.2.2 Electrical conductivity (EC) determination

For determination of electrical conductivity (EC), 20g of soil were mixed with 20ml of water to make a 1:1 soil:water suspension. The suspension was stirred for 30 minutes, and EC readings taken directly from the settled suspension using an electrical conductivity meter, and expressed as deci-Siemens per metre (dS/m).

3.2.3 Organic carbon determination

Soil organic carbon was determined by the wet digestion method of Walkley and Black as described by Nelson and Sommers (1982). One gram sample was placed into a 250ml

Erlenmeyer flask, mixed with 10ml $K_2Cr_2O_7$ solution and 20ml of concentrated H_2SO_4 and swirled for one minute. It was left to settle for 30 minutes. One hundred ml of distilled water were added to the mixture, followed by 10ml of conc. phosphoric acid and 1ml of diphenylamine indicator, and titrated against 1M ferrous ammonium sulphate. The amount of $K_2Cr_2O_7$ reduced was used to calculate the soils' organic carbon content.

3.2.4 Phosphorus determination

Phosphorus determination for some samples was determined by the Olsen and some by the Bray and Kurtz method. For soils with pH lower than 6.5, phosphorus determination using Bray and Kurtz method was carried out as described by Murphy and Riley (1962). Three g of sample were mixed with 35ml of extracting solution (NH^+_4 + HCl), shaken for one minute, then filtered. Five ml of filtrate were mixed with boric acid then

made to 50ml. Colour was developed using a mixture of ascorbic acid and ammonium molybdate and the concentration of P in the samples calculated after taking absorbance readings on a spectrophotometer at 890nm, with appropriate standards.

For soils of pH 7.5 or above, phosphorus determination by Olsen method was carried out as described by Watanable and Olsen (1965). Five g of sieved soil were weighed in a 250ml plastic bottle. Then 100ml 0.5M $NaHCO_3$ added, shaken in a rotating shaker for exactly 30 minutes and filtered immediately. Five ml of filtrate were transferred to a 50ml volumetric flask and five ml of 0.3M H_2SO_4 were added. The mixture was shaken for 30 minutes. Thirty ml of distilled water were added, followed by 10ml of ammonium molybdate - ascorbic acid solution. The mixture was made to the volume using distilled water. Absorbance was read at 890nm and the amount of phosphorus calculated.

3.3.5 Total nitrogen

Total nitrogen was determined using the Kjeldahl method as described by Bremner and Mulvaney (1982). One g of sample was digested for two hours, cooled and the digest made up to 50ml using distilled water. Five

ml of the diluted digest were distilled in the presence of 40% NaOH. The NH_3 gas produced was trapped in boric acid as indicator, then titrated with 0.05M sulphuric acid until colour changed from green to light red. The titre was used to calculate the soil's total nitrogen content.

3.2.6 Determination of exchangeable bases

Exchangeable bases were determined as follows: A fifty-g sample was mixed with NH_4OAc (pH 7.0) solution, stirred and filtered. The residue was washed by passing NH_4OAc solution until the volume was just below the 100ml mark of the collecting volumetric flask. It was then made to the mark by addition of NH_4OAc (National Soil Service, 1987). This solution was used to determine exchangeable cations using atomic absorption spectrophotometry.

3.2.7 Particle size distribution

Particle size analysis was determined by the Bouyoucos hydrometer method (National Soil Service, 1987). 50g samples were mixed with H_2O_2, HCl and sodium hexametaphosphate and shaken for two hours to disperse the soil particles. The suspension was poured into a one litre

sedimentation cylinder and hydrometer readings taken at the prescribed times. The percentages of sand, silt and clay were calculated, and texture determined using the USDA textural triangle. Temperatures were recorded to allow correction of the readings.

3.3 Brief description of the soils' properties

The properties of soil samples used in the study are given in Table 1. The EC ranged from 0.1 to 36.7dS/m, which may be ranked as being low, medium to high levels. pH values were on alkaline range, as they ranged from 7.27 to 10.11, Exchangeable Ca, Mg, Na and K respectively ranged from 10.1 to 122, 0.5 to 16.1, 0.9 to 88.3 and 2 to 56 Mcq/100g. The high Ca levels are due to the soils being saline, and therefore having high levels of Ca. The % total nitrogen, % organic carbon and extractable phosphorus (ppm) ranged from 0.02 to 0.04, 0.78 to 1.84 and 0.02 to 6.08 respectively. The textural classes were clay and clay loam.

Table 1. Some physical and chemical properties of soils used in the study

Location	EC (dS/m)	pH	Ca (Meq/ 100g)	Mg (Meq/ 100g)	Na (Meq/ 100g)	K (Meq/ 100g)	%N	%OC	P(ppm)	Textural class
Mkata off road	0.1	7.27	8.5	6.4	0.9	2	0.02	1.6	3.3	Clay
Mkata road side	0.45	7.65	10.1	6.6	1.1	3 5	0.02	0.78	4.67	Clay
Kikundi Morogoro town, bottom layer	4.6	10 11	5.5	0.5	47.1	5.6	0.02	1.84	0.22	Clay loam
Kikundi upper 5cm layer	7.27	9.91	13.9	0.5	44.1	5.4	0.03	1.56	3.1	Clay loam
Near SUA main gate	10.9	7.88	8.3	12.9	16.9	2.3	0.04	1.82		Clay
Chalinze	19.7	7.32	4.2	11.9	45.8	2.3	0.02	0.91	0.42	Clay loam
Chalinze top layer	36.0	9.88	19	10.8	88.3	1.5	0.03	1.35	0.18	Clay loam
Catholic Capuchin Seminary	36.7	7.49	12.2	16.1	35.3	1.4	0.03	1.01	6.08	Clay

3.4 Enumeration of *Streptomyces*

The plate count method was used to enumerate *Streptomyces* in the soils sampled. The medium used was starch casein agar (Küster and Williams, 1964; Williams and Wellington, 1982; Wollum, 1982) fortified with penicillin G and actidione

(cycloheximide) to suppress development of simple bacteria and fungi, respectively (Williams and Davies, 1965; Porter *et al.*, 1965).

In view of the fact that soils with different levels of salinity were collected, it was vital to maintain similar levels of salinity in culture media. Therefore, the starch casein agar was further modified to attain the different levels of salinity found in the soils, as follows. A large volume of 1:1 soil: water extract was prepared using the soil from the Morogoro Catholic Capuchin Seminary which had an EC of 35dS/m. Portions of this extract with EC of 35dS/m were diluted using distilled water to obtain solutions simulating the levels of salinity of each of the soils sampled. These solutions were then used to suspend/dissolve the ingredients of starch casein agar to obtain media which simulated the soils' salinity conditions. They were sterilised in an autoclave at 121°C and 15 pounds

per square inch for 15 minutes. These media were used to culture *Streptomyces* from soils with respective salinity levels for purposes of enumerating the *Streptomyces.*

Ten g of soil (oven dry equivalent) were weighed in four replicates and placed into bottles containing 90ml of sterile water, making the 10^1 soil suspension. Then serial dilution to make 10^2, 10^3, 10^4, and 10^5 were made and plated in media of respective EC values. The plates were incubated at 25 to 28^{0}C for 14 days and *Streptomyces* counting was made on the plates showing a good distribution of *Streptomyces* colonies. The colony counts were converted to *Streptomyces* populations per gram of oven dry soil, and the data were transformed to the logarithmic scale (base 10). The data were analysed for variance using the completely randomised design to evaluate the effects of salinity on *Streptomyces* populations. For all the samples regression analysis was carried out to evaluate the relationships between the *Streptomyces* populations and different soil characteristics.

3.5 Isolation of *Streptomyces*

The isolation of *Streptomyces* colonies was done in a lamina flow cabinet sterilised for one hour by U.V radiation from an inbuilt ultra violet lamp.

Streptomyces colonies from the starch - casein agar plates from section 3.4 were picked using a sterile inoculating loop and transferred aseptically to plates containing oatmeal agar (Küster, 1959; Shirling and Gottlieb, 1966) prepared to the different EC values as was described for starch casein agar in section 3.4. The plates were incubated for 14 days for the colonies to grow. This step was repeated to obtain colonies which were free from contamination. These colonies or isolates were used for further studies as described in sections 3.6 to 3.9 below.

3.6 Determination of colour of the *Streptomyces* isolates' aerial mycelium

Preliminary characterisation of the *Streptomyces* isolates was done by determining their colours. The colour of aerial mature sporulating mycelium were determined after transferring the *Streptomyces* colonies to oatmeal agar (Küster, 1959) as described in section 3.5. Colour determination was done by assigning the closest colour names using the colours compiled by Pridham (1964) due to inavailability of more appropriate methods.

3.7 Determination of morphology of the isolates' spore chains

A sterile isolation loop was used to pick a small peripheral portion of a well-grown mature part of a colony, which was placed on microscope slide. The Nikon 98455 light microscope was used to observe the morphology as described by Shirling and Gottlieb (1966), using a magnification of 600x. The morphology characterisation was done by comparing and assigning one of the classes described by Pridham *et al.* (1958), Shirling and Gottlieb (1966), and Brock *et al.* (1994). Several fields were examined to confirm a morphological class.

3.8 Evaluation of range of tolerance of *Streptomyces* to salinity

Oatmeal agar media of different electrical conductivity to simulate the EC values of original soils were made from soil extracts as described in section 3.4. The solutions had electrical conductivity of 0, 4, 8, 12, 16, 20, 24, 30 and 35dS/m. Also, solutions of 2, 5, 8 and 10 % salt concentration were prepared. Then the *Streptomyces* isolates were grown on the media prepared using the above solutions to evaluate the range of salinity tolerance. All isolates were grown in all levels of salinity.

3.9 Evaluation of ability of *Streptomyces* isolates to inhibit growth of plant pathogenic bacteria

Streptomyces isolates were taken from the oatmeal agar plates and aseptically transferred to nutrient agar plates. A straight line of *Streptomyces* innoculum was streaked across the agar medium in a petri dish. The plates were incubated for three days at 25 - 28°C. Test organisms were then streaked at right angles to the *Streptomyces* straight-line colony as described by Prescott and Dunn (1959), Alexander (1983) and Brock *et al.* (1994). The test organisms were the plant pathogens *Xanthomonas phaseoli, X. oryzae pv.* oryzae, *Clavibacter michiganensis sub sp. Michiganensis, X. vascatoria, X. phaseolicoli var* fuscoris and *Acidovorax avenae.* A description of the characteristics of the test plant pathogens is given in Table 2.

These bacteria were then cultured in nutrient broth at 25°C for three days to provide inoculum for streaking at right angles to the *Streptomyces* colony. The plates were incubated for a further three to seven days. The extent of inhibition of pathogen growth by *Streptomyces* was recorded by measuring the length of the inhibited zone, in mm.

However, before streaking the test bacteria, each of them was first grown in nutrient agar under different pH conditions to examine their ability to grow at those different pH levels. With the exception of *Acidovorax avenae*, the rest of the pathogenic bacteria were able to grow at the pH values simulating the pH conditions of the soils of this study.

Table 2. Some characteristics of the plant pathogenic bacteria tested.

Organism	Gram reaction	Significance/importance
Acidovorax avenae	-	Causes bacterial leaf blight
C. michiganensis sub sp. michiganensis	-	causes bacterial canker of tomato
X. oryzae pv oryzae	-	Causes bacterial blight rice
X. vascatoria	-	Causes leaf spot of tomato
X. phaseolicoli var fuscoris	-	Causes common bacterial blight of beans
X. phaseoli	-	Causes bean blight

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Influence of soil salinity on populations of *Streptomyces*

The populations (expressed as $\log_{10}$) of *Streptomyces* isolated from soils of different levels of salinity are presented in Table 3.

There were significant differences (P = 0.05) in the populations of *Streptomyces* between some soils. Generally, *Streptomyces* populations were higher in soils of lower salinity (lower EC), and the vice versa. For example, normal soils, those with EC lower than 0.45dS/m, had populations ($\log_{10}$) of 3.7 to 4.9 per gram of soil, while at EC around 36dS/m the populations ($\log_{10}$) were 2.0 to 2.3 per gram of soil.

The differences in the populations of *Streptomyces* in different soils may be ascribed to salinity. *Streptomyces* are involved in processes like decomposition of organic matter to release the nutrients necessary for plant growth, and products of decomposition improve soil physical properties such as aeration, water retention capacity and chemical

properties like cation exchange capacity and buffering capacity (Singh *et al.*, 1993). It may be inferred that lower populations of *Streptomyces* as can be found in salt affected soils of the present studies will reduce organic matter decomposition and consequently affect some of the properties listed above. For example, Singh *et al.* (1993) reported that

Table 3. Populations of *Streptomyces* isolated from soils having different levels of salinity

Location	Electrical conductivity (EC)	Populations of *Streptomyces* (per gram of soil)
	(dS/ m)	($\log_{10}$)
Chalinze Mzee, top 5 cm layer	36.0	2.0d ± 0
Morogoro, near Catholic Capuchin Seminary	36.7	2.3d ± 0.44
Kikundi, Morogoro town, upper 5 cm layer	7.27	2.5cd ± 0.37
Chalinze Mzee 5 - 10 cm	19.7	3.1c ± 0.12
Mkata off-road	0.1	3.7b ± 0.23
Kikundi, 5 - 10 cm bottom layer	4.6	3.7b ± 0.3
SUA farm, near SUA main gate main gate	10.9	4.7a ± 0.12
Mkata roadside	0.45	4.9a ± 0.25

Means of *Streptomyces* populations within the same column followed by the same letter were not significantly different (P = 0.05) according to Duncans' New Multiple Range Test.

irrigation of soils using saline water (EC = 5dS/m) reduced the rates of carbon and nitrogen mineralization. Kontchou and Blondeau (1992) reported that the presence of sodium salts in the soil reduced degradation of soil humic acid by *Streptomyces viridosporus*.

The lower populations of *Streptomyces* observed in the soils of higher salinity may have been caused by interference by the salts in physiological processes of *Streptomyces* such that most isolates which were not adapted to salts failed to grow. Band *et al.* (1992) reported that microorganisms growing in salt affected soils converted polysaccharides in their cells to soluble molecules which were used for osmoregulation. Consequently, physiological processes were interfered with and the microorganisms failed to grow, resulting in low populations as observed in the present studies. Kontchou and Blondeau (1992) reported that presence of salts, for example Na salts, affected oxidative metabolic processes of *Streptomyces viridosporus*. The higher populations observed presently in some saline soils, for example soils near SUA main gate, with salinity level of 10.9dS/m and *Streptomyces* populations ($\log_{10}$) of 4.7 per gram of soil, suggests that the *Streptomyces* isolates at that location were well adapted

Means of *Streptomyces* populations within the same column followed by the same letter were not significantly different (P = 0.05) according to Duncans' New Multiple Range Test.

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to the saline environment. The mechanism of adaptation is not well understood at present, and needs to be investigated.

The *Streptomyces* populations from normal soils observed in this study are comparable with those reported by Ikerra (1986), Keya (1998), Philip (1998) and Ndonde (1998). The *Streptomyces* populations (log_{10}) in the studies just cited ranged from 4.6 to 5.29 per gram of soil, while the populations observed in the present study ranged from 3.7 to 4.9 per gram of soil. This agreement may imply that the observed populations are a reflection of the levels of populations of *Streptomyces* prevailing in the soils during the rainy seasons as was sampled in all these studies. The *Streptomyces* populations in the more saline soils of the present studies are comparable with those reported else where by Goodfellow and Hyenes (1983) as cited by Goodfellow and Williams, (1983). Those reported by Goodfellow and Hyenes (1983) as cited by Goodfellow and Hyenes (1983) were around 2.24 per gram of soil (on the log_{10} scale), as was 2.0 to 2.3 per gram of soil in the present study. This implies that salinity affects some universally fundamental physiological process(es).

4.2 Relationships between *Streptomyces* populations and some soil characteristics

Table 4 shows regression parameters of *Streptomyces* populations on selected soil characteristics. There was a significant (P = 0.05) positive correlation between *Streptomyces* populations and total nitrogen in soil. There was a significant (P = 0.05) negative correlation between *Streptomyces* populations and salinity (EC) levels, meaning that as the level of salinity increased, the populations of *Streptomyces* decreased as was observed in section 4.1. However, regression of *Streptomyces* populations on organic carbon, exchangeable bases, pH, silt, sand and clay were not significant. The positive correlation of populations with total nitrogen may be related to the fact that nitrogen is required by microorganisms for protein synthesis (Alexander, 1983). Similar results were reported by Kamata *et al.* (1997) that higher populations of *Streptomyces* were encountered in soils having large quantities of nitrogen.

The negative correlation of *Streptomyces* populations and salinity may have been caused by interference by the salts in some physiological processes of the *Streptomyces* (Goodfellow and Hyenes (1983) as cited by

Goodfellow and Hyenes (1983) as already discussed in section 4.1.

The lack of correlation between *Streptomyces* populations and the rest of the soils' characteristics may imply that for the present soils, those characteristics did not vary greatly to the extent of affecting the populations profoundly. On the contrary, significant relationships between *Streptomyces* populations and pH, organic carbon and clay were reported by Ndonde (1998). The soils used by Ndonde (1998) exhibited a wider degree of variability of soil characteristics than was present in the soils of the present study.

Table 4. Regression between number of *Streptomyces* and different soil parameters.

Parameter	R^2	P Value	NS
Exchangeable K	0.011	0.74	NS
Exchangeable Na	0.007	0.83	NS
Exchangeable Mg	0.036	0.64	NS
Exchangeable Ca	0.057	0.85	NS
Sum of bases	0.366	0.78	NS
%Total N	0.633	0.01	*
% Organic carbon	0.696298	0.52	NS
% Clay	0.00294523	0.89	NS
% Silt	0.00133075	0.93	NS
% Sand	0.00364407	0.88	NS
pH	0.139429	0.36	NS
EC	0.57578	0.02	*

NS = Not significant

* = Significant

4.3 Characterisation of *Streptomyces* isolates according to colour of aerial mycelium and morphology of spore chains

The colours of aerial mycelia and morphologies of spore chains of the *Streptomyces* isolates are presented in Table 5. Different categories of colour and morphology were observed, signifying, in both parameters, a wide range of variability in the isolates. The summaries of the distribution of these colours and morphological classes are given in sections 4.3.1 and 4.3.2, respectively.

Table 5. Colours and morphologies of the *Streptomyces* isolates.

Isolate	Morphology of spore chains	colour of aerial mycelium
0.1(1)	Straight	Gray
0.1(2)	Straight	Gray
0.1(3)	Fascicled	White gray
0.1(4)	Straight	White gray
0.1(5)	Straight	White gray
0.1(6)	Straight	Grayish
0.45(1)	Flexuous	Reddish white
0.45(2)	Flexuous	Reddish white
0.45(3)	Flexuous	Grayish
0.45(4)	Closed spirals	Grayish
0.45(5)	Closed spirals	Reddish gray
0.45(6)	Flexuous	Grayish
0.45(7)	Flexuous	Reddish gray
0.45(8)	Hooks	Gray
0.45(9)	Open spirals	Bluish
4.6(1)	Flexuous	Whitish
4.6(2)	Flexuous	Whitish
4.6(3)	Flexuous	Whitish
4.6(4)	Flexuous	Whitish
4.6(5)	Straight	Whitish
4.6(6)	Flexuous	Whitish gray
4.6(7)	Straight	Grayish
4.6(8)	Straight	Whitish gray
4.6(9)	Straight	Whitish gray
4.6(10)	Straight	Whitish gray
4.6(11)	Straight	Whitish gray
7.29(1)	Straight	Gray
7.29(2)	Straight	Gray

7.29(3)	Flexuous	Gray
7.29(4)	Monoverticillate	Gray
10.9(1)	Open loops	Gray
10.9(2)	Flexuous	Gray
10.9(3)	Straight	White gray
10.9(4)	Flexuous	White gray
10.9(5)	Straight	White gray
10.9(6)	Open loops	Grayish
10.9(7)	Straight	Whitish gray
10.9(8)	Flexuous	Whitish gray
10.9(9)	Flexuous	Whitish gray
10.9(10)	Flexuous	Grayish
10.9(11)	Flexuous	Grayish
10.9(12)	Straight	Reddish
10.9(13)	Flexuous	Grayish
10.9(14)	Flexuous	Whitish gray
10.9(15)	Open loops	Gray
10.9(16)	Straight	Red gray
10.9(17)	Open loops	Grayish
10.9(18)	Flexuous	Grayish
10.9(19)	Flexuous	Grayish
10.9(20)	Flexuous	Whitish
10.9(21)	Flexuous	Whitish gray
10.9(22)	Flexuous	Red grayish
10.9(23)	Flexuous	Gray
10.9(24)	Open loops	Gray
10.9(25)	Open spirals	Reddish white
10.9(26)	Open loops	Grayish
10.9(27)	Flexuous	Grayish
10.9(28)	Flexuous	Whitish gray
10.9(29)	Straight	Grayish
10.9(30)	Flexuous	Gray
10.9(31)	Flexuous	Whitish gray
10.9(32)	Flexuous	Reddish gray
10.9(33)	Flexuous	Gray
10.9(34)	Spiral	Grayish
10.9(35)	Flexuous	Whitish gray
10.9(36)	Flexuous	Gray
10.9(37)	Open spirals	White
10.9(38)	Flexuous	Red brown
10.9(39)	Flexuous	White
10.9(40)	Flexuous	Reddish
10.9(41)	Flexuous	Grayish
10.9(42)	Flexuous	Reddish
10.9(43)	Flexuous	Whitish
10.9(44)	Straight	Reddish
10.9(45)	Open loops	Whitish gray
10.9(46)	Flexuous	Whitish gray
10.9(47)	Flexuous	Whitish
10.9(48)	Flexuous	White
10.9(49)	Flexuous	Whitish gray
19.7(1)	Open spirals	Gray
19.7(2)	Flexuous	Gray
19.7(3)	Flexuous	Gray
19.7(4)	Flexuous	Grayish
35(1)	Open loop	Grayish
35(2)	Open loop	Grayish
35(3)	Open loop	Grayish
35(4)	Open loop	Grayish
35(5)	Open loop	Grayish
35(6)	Open loop	Grayish
35(7)	Open loop	Grayish
35(8)	Closed spiral	Grayish

35(9)	Straight	Grayish
35(10)	Open loop	Grayish
35(11)	Open spiral	Grayish
35(12)	Flexuous	Grayish
35(13)	Open loop	Grayish
35(14)	Open loop	Grayish
35(15)	Flexuous	Gray
35(16)	Flexuous	Gray
35(18)	Closed spirals	Gray
35(19)	Closed spiral	Grayish
35(20)	Flexuous	Grayish
35(21)	Open loops	Grayish
35(22)	Flexuous	Whitish
35(23)	Open spiral	Grayish
35(24)	Open spiral	Gray
35(25)	Flexuous	Gray
35(26)	Open spiral	Gray
35(27)	Straight	Grayish
35(28)	Straight	Grayish
35(29)	Open loop	Grayish
35(30)	Open loop	Whitish
35(31)	Straight	Grayish
35(32)	Open loop	Gray
35(33)	Open loop	Grayish
35(34)	Open loop	Whitish gray
35(35)	Open loop	Whitish gray

4.3.1 Distribution of *Streptomyces* according to colours of their aerial mycelium

The distributions of the *Streptomyces* isolates according to colours of aerial mycelia are presented in Table 6. The classes of colours of the isolates ranged from gray to white to red types and their intergrades, in different proportions. The gray series was the most dominant.

Table 6. Summary of distribution of *Streptomyces* isolates according to colour of aerial mycelium.

Colour	Number of isolates	Abundance (%)
Grayish	41	34.75
Gray	25	21.2
Whitish gray	21	17.80
Whitish	10	8.47
White gray	6	5.08
Reddish	4	3.39
Reddish gray	3	2.5
White	2	1.69
Reddish white	2	1.69
Red brown	1	0.8
Red grayish	1	0.8
Reddish white	1	0.8
Dark gray	1	0.8

The different classes of colours as observed in the present studies give an indication of genetic diversity or variability that exists in the *Streptomyces* flora of these soils. However, methods of colour designation in *Streptomyces* have not been standardised internationally (Pridham, 1965). This may present problems in trying to use colour as a major criterion in species designation, and, therefore, any variability observed in the present studies may not necessarily imply variability in species.

4.3.2 Distribution of *Streptomyces* isolates according to morphologies of spore chains

The distribution of *Streptomyces* isolates according to morphologies of their spore chains is presented in Table 7. The distribution of *Streptomyces* isolates according to the morphology of aerial mycelium ranged from straight to flexuous to spirals, in different proportions. The flexuous morphological type was dominant, followed, equally, by the open loop and the straight types.

Table 7. Summary of distribution of *Streptomyces* isolates according to morphology of spore chains.

Morphology	Number of isolates	Abundance (%)
Flexuous	52	44.4
Open loop	24	20.5
Straight	24	20.5
Open spiral	8	6.8
Closed spiral	5	4.3
Fascicled	1	0.8
Monovertici llate	1	0.8
Hooks	1	0.8
Spiral	1	0.8

As in the case of colour of aerial mycelium, the different classes of morphology of spore chains may likewise be an indication of diversity of *Streptomyces* isolated from the soils of this study.

4.3.3 Distribution of colour of *Streptomyces* isolates according to level of salinity

The distributions of colours of the *Streptomyces* isolates according to level of soil salinity are presented in Table 8.

Table 8. Distribution of colours of *Streptomyces* isolates according to level of soil salinity.

Level of salinity (EC)	Colour of aerial mycelium	Number of isolates	% Distribution within salinity level
- - -(dS/m)- - -			
0.1	Gray	2	33.3
	White gray	3	50
	Grayish	1	16.7
0.45	Reddish white	2	22.2
	Graysh	3	33.3
	Reddish gray	2	22.2
	Gray	1	11.1
	Bluish	1	11.1
4.6	Whitish	5	45.5
	Whitish gray	5	45.5
	Grayish	1	9
7.29	Gray	4	100
10.9	Gray	8	16.3
	White gray	3	6.1
	Grayish	12	24.5
	Whitish gray	11	22.4
	Reddish	4	2
	Red gray	1	2
	Whitish	3	6.1
	Red grayish	1	2
	Reddish white	1	2
	Reddish gray	1	2
	White	3	6.1
	Red brown	1	2
19.7	Gray	3	75
	Grayish	1	25

35	Grayish	22	62.9
	Gray	7	20
	Whitish	2	5.7
	Whitish gray	2	5.7

Different colour classes were observed in soils of different levels of salinity. In soils of salinity levels of 10.9(dS/m), for example, ten colour classes were observed, viz.; gray, white gray, grayish, whitish gray, reddish, red gray, whitish, red grayish, reddish white, reddish gray, white and red brown. Greyish colours were the most dominant (24.5%) followed by whitish Gary (22.4%). The red series constituted 6% and was the least dominant. Variations were similarly observed in the other levels of salinity. It is difficult to conclude whether these variations represent systematic or random trends. The reasons for such variations are not well understood, but could well be a response to some environmental conditions.

4.3.4 Distribution of Morphology of *Streptomyces* according to level of salinity

The distribution of morphological classes with soil salinity is shown in Table 9.

Table 9. Distribution of morphology according to level of salinity.

Level of salinity (EC dS/m)	Morphology of spore chains	Number of isolates	(%) of isolates within salinity level.
0.1	Straight	5	83.3
	Fascicled	1	16.7
0.45	Flexuous	5	55.5
	Closed spiral	2	22.2
	Hooks	1	11.1
	Open spirals	1	11.1
4.6	Flexuous	5	45.5
	Straight	6	55.5
7.29	Straight	2	50
	Flexuous	1	25
	Monovert icillate	1	25
10.9	Open loops	7	14.3
	Flexuous	32	65.3
	Straight	6	12.2
	Open spirals	2	6.1
	Spiral	1	2
19.7	Flexuous	3	75
	Open	1	25

	spiral		
35	Open	16	45.7
	loop		
	Closed	5	14.3
	spiral		
	Straight	4	11.4
	Open	4	11.4
	spiral		
	Flexuous	6	17.1

Different morphological types were observed in all locations. Soils of salinity levels of 10.9 and 35dS/m harboured *Streptomyces* isolates of five different morphological categories each. In soils of EC 10.9dS/m, for example, the following morphological categories were observed: Flexuous (65.3%), open loops (14.3%), straight (12.2%), open spirals (6.1%) and spiral (2%). Variations were similarly observed in the other levels of salinity. As already mentioned, it is hard to speculate at present whether these variations with levels of salinity are systematic or random.

4 3.5 Range of tolerance of the isolated *Streptomyces.*

All the isolates were able to grow well on all the levels of salinity up to 35dS/m (<2% salt concentration) (Table 10). Some *Streptomyces* isolates grew, to different extents, on media containing 2%, 5% or 8% salt concentration. None of the isolates grew at 10% salt concentration (Table 7).

The soils of the present study, with less than two per cent salt concentration, represent a range of low salt concentration for microorganisms. It shows that the soils' differences in salinity were within physiological versatility of the tested *Streptomyces* isolates, that even the higher salt concentrations were not high enough to upset the physiological processes of the *Streptomyces* isolates. Therefore the *Streptomyces* isolates were well adapted to such saline environments. Watson and Williams (1973) reported tolerance to salinity by some *Streptomyces* strains. The *Streptomyces* strains they tested for range of salinity tolerance were able to grow on media with up to 1.23M NaCl concentration (which is 7.2% salt concentration).

For the case of the *Streptomyces* isolates which grew differently on media of 2% salt concentration and above, it can be inferred that those *Streptomyces* isolates could tolerate medium to extremely high salt concentrations. The fact that some *Streptomyces* species do not tolerate certain ranges of salt concentrations have been reported by Wellington and Toth (1994). A similar phenomenon was reported by Watson and Williams (1973) that some *Streptomyces* which grew on media of 0.41M NaCl concentration could not do so on media of higher NaCl concentration such as 0.82 or 1.23M, and that none grew on media of 1.64M NaCl concentration. The differences in tolerance to salts observed in the present studies reflect differences in adaptation that exists between the *Streptomyces* isolates.

Table 10. Growth of *Streptomyces* isolates at different levels of salt concentration.

	Growth				
Isolate	<2% salt concentration	2% salt concentration	5% salt concentration	8% salt concentration	>10% salt concentration
0.1(1)	Good	Good	Good	Poor	Nil
0.1(2)	Good	Fairly good	Fairly good	Poor	Nil
0.1(3)	Good	Good	Good	Poor	Nil
0.1(4)	Good	Good	Good	Poor	Nil
0.1(6)	Good	Fairly good	Fairly good	Fairly good	Nil
0.45(1)	Good	Good	Good	Good	Nil
0.45(2)	Good	Good	Good	Good	Nil
0.45(3)	Good	Good	Good	Poor	Nil
0.45(4)	Good	Fairly good	Fairly good	Poor	Nil
0.45(5)	Good	Good	Good	Good	Nil
0.45(6)	Good	Good	Good	Good	Nil
0.45(7)	Good	Good	Good	Good	Nil
4.6(1)	Good	Good	Good	Good	Nil
4.6(2)	Good	Good	Fairly good	Fairly good	Nil
4.6(4)	Good	Fairly good	Fairly good	Fairly good	Nil
4.6(5)	Good	Fairly good	Fairly good	Fairly good	Nil

4.6(6)	Good	Fairly good	Fairly good	Fairly good	Nil
4.6(7)	Good	Fairly good	Fairly good	Fairly good	Nil
4.6(8)	Good	Fairly good	Fairly good	Fairly good	Nil
4.6(9)	Good	Good	Good	Good	Nil
4.6(11)	Good	Fairly good	Fairly good	Fairly good	Nil
7.29(1)	Good	Good	Good	Good	Nil
7.29(2)	Good	Good	Good	Good	Nil
7.29(3)	Good	Good	Good	Poor	Nil
7.29(4)	Good	Good	Good	Poor	Nil
10.9(1)	Good	Good	Good	Good	Nil
10.9(2)	Good	Good	Good	Good	Nil
10.9(3)	Good	Good	Good	Good	Nil
10.9(4)	Good	Good	Good	Good	Nil
10.9(5)	Good	Good	Good	Good	Nil
10.9(6)	Good	Good	Good	Good	Nil
10.9(7)	Good	Good	Good	Good	Nil
10.9(8)	Good	Good	Good	Good	Nil
10.9(10	Good	Good	Good	Good	Nil
10.9(11	Good	Good	Good	Good	Nil
10.9(12	Good	Good	Good	Good	Nil
10.9(13	Good	Good	Good	Good	Nil
10.9(14	Good	Good	Good	Good	Nil
10.9(15	Good	Good	Good	Good	Nil
10.9(16	Good	Good	Good	Good	Nil
10.9(17	Good	Good	Good	Good	Nil
10.9(19	Good	Good	Good	Good	Nil
10.9(20	Good	Good	Good	Good	Nil
10.9(21	Good	Good	Good	Good	Nil
10.9(22	Good	Good	Good	Good	Nil
10.9(23	Good	Good	Good	Good	Nil
10.9(24	Good	Good	Good	Good	Nil
10.9(25	Good	Good	Good	Good	Nil
10.9(26	Good	Good	Good	Good	Nil
10.9(27	Good	Good	Good	Good	Nil
10.9(28	Good	Good	Good	Good	Nil
10.9(29	Good	Good	Good	Good	Nil
10.9(30	Good	Good	Good	Good	Nil
10.9(31	Good	Good	Good	Good	Nil
10.9(32	Good	Good	Good	Good	Nil
10.9(33	Good	Good	Good	Good	Nil
10.9(34	Good	Good	Good	Good	Nil
10.9(35	Good	Good	Good	Good	Nil
10.9(36	Good	Good	Good	Good	Nil
10.9(37	Good	Good	Good	Good	Nil
10.9(38	Good	Good	Good	Good	Nil
10.9(39	Good	Good	Good	Good	Nil
10.9(40	Good	Good	Good	Good	Nil
10.9(41	Good	Good	Good	Good	Nil
10.9(42	Good	Good	Good	Good	Nil
10.9(43	Good	Good	Good	Good	Nil
10.9(44	Good	Good	Good	Good	Nil
10.9(45	Good	Good	Good	Good	Nil
10.9(46	Good	Good	Good	Good	Nil
10.9(47	Good	Good	Good	Good	Nil
10.9(48	Good	Good	Good	Good	Nil
10.9(49	Good	Good	Good	Good	Nil
35((1)	Good	Good	Good	Fairly good	Nil
35(2)	Good	Good	Fairly good	Fairly good	Nil
35(3)	Good	Good	Good	Fairly good	Nil
35(4)	Good	Good	Fairly good	Fairly good	Nil

35(5)	Good	Good	Good	Fairly good	Nil
35(6)	Good	Good	Good	Fairly good	Nil
35(7)	Good	Good	Good	Good	Nil
35(8)	Good	Good	Good	Fairly good	Nil
35(9)	Good	Good	Good	Good	Nil
35(10)	Good	Fairly good	Fairly good	Fairly good	Nil
35(11)	Good	Fairly good	Fairly good	Fairly good	Nil
35(12)	Good	Fairly good	Fairly good	Fairly good	Nil
35(13)	Good	Good	Good	Fairly good	Nil
19.7(1)	Good	Good	Fairly good	Poor	Nil
19.7(2)	Good	Fairly good	Poor	Poor	Nil
19.7(3)	Good	Fairly good	Fairly good	Poor	Nil
19.7(4)	Good	Fairly good	Fairly good	Poor	Nil
19.7(5)	Good	Good	Poor	Poor	Nil
19.7(6)	Good	Good	Good	Fairly good	Nil

4.4 Ability to produce antibiotics

4.4.1 General

The extent of antibiosis (mm) of inhibited zone is presented in Table 11. Some isolates inhibited growth of some plant pathogens to different extents. For example, isolate 0.45(1) showed 12mm inhibition zone on medium with EC zero against *Xanthomonas phaseoli*, while isolate 0.45(3) showed > 45mm against the same pathogen on the same medium. Isolate 7.29(1) showed 19mm against *Clavibacter michiganensis* sub sp. michiganensis on medium of EC zero while isolate 0.45(3) showed > 45mm against the same pathogen on the same medium.

Some isolates for example 7.29(1), and 10.9(21) showed strong antibiosis (>25mm of

inhibition zone), others showed moderate antibiosis (11 - 25mm) while others showed weak antibiosis (<11mm). Others did not inhibit at all the test pathogens, for example, isolates 10.9(42) and 35(8). The pathogen *Acidovorax avenae* was not inhibited by any of the *Streptomyces* isolates tested.

Table 11. Extent of antibiosis indicated by zone of inhibition (in mm) shown by *Streptomyces* isolates at different salt concentrations.

Isolate	Pathogen					
	Xanthomonas phaseoli	*X. Oryzae* pv oryzae	*Clavibacter michiganensis* sub sp michiganensis	*X. vascatoria*	*X. phaseolicoli* var fuscoris	*Acidovorax avenae*
0.45(1)*	12	0	0	9	0	0
0.45(1)**	0	0	0	0	0	0
0.45(2)*	7	0	>45	0	0	0
0.45(2)**	0	0	0	0	0	0
0.45(3)*	>45	0	0	0	0	0
0.45(3)**	>45	0	0	0	0	0
0.45(4)*	0	0	0	0	0	0
0.45(4)**	0	0	0	0	0	0
0.45(5)*	0	0	0	0	0	0
0.45(5)**	0	0	0	0	0	0
0.45(6)*	0	0	0	5	0	0
0.45(6)**	0	0	0	5	0	0
0.45(8)*	0	0	0	0	0	0
0.45(8)**	0	0	29	0	0	0
7.29(1)*	14	0	15	0	9	0
7.29(1)**	0	0	19	0	0	0
10.9(1)*	0	0	0	11	9	0
10.9(1)**	0	0	29	10	0	0
10.9(4)*	9	0	19	0	0	0
10.9(4)**	0	0	19	0	0	0
10.9(5)*	0	0	15	10	0	0
10.9(5)**	0	0	27	0	0	0
10.9(6)*	0	0	26	11	16	0
10.9(6)**	0	0	15	10	8	0
10.9(6)***	0	0	0	0	0	0
10.9(7)*	0	0	0	0	0	0
10.9(7)**	0	0	0	0	0	0
10.9(13)*	0	0	0	0	0	0
10.9(13)**	0	0	14	0	0	0
10.9(15)*	0	0	17	0	11	0
10.9(15)**	0	0	15	0	7	0

10.9(15)***	0	0	0	0	0	0
10.9(16)*	0	0	0	0	0	0
10.9(16)**	0	0	0	0	0	0
10.9(19)*	0	0	0	0	0	0
10.9(19)**	0	0	>45	0	0	0
10.9(21)*	>45	9	17	0	0	0
10.9(21)**	12	0	10	0	0	0
10.9(21)***	0	0	0	0	0	0
10.9(22)*	0	0	0	0	0	0
10.9(22)**	0	0	26	0	0	0
10.9(26)*	13	0	29	12	11	0
10.9(26)**	14	0	12	0	9	0
10.9(26)***	15	0	22	0	0	0
10.9(27)*	5	0	5	0	0	0
10.9(27)**	0	0	26	0	0	0
10.9(35)*	16	0	16	14	14	0
10.9(35)**	0	0	10	0	11	0
10.9(37)*	7	0	0	0	0	0
10.9(37)**	0	0	19	0	0	0
10.9(40)*	0	0	0	7	0	0
10.9(40)**	0	0	0	0	0	0
10.9(42)*	0	0	0	0	0	0
10.9(42)**	0	0	0	0	0	0
10.9(46)*	14	0	0	0	0	0
10.9(46)**	14	0	>45	0	0	0
35(1)*	10	0	20	11	>45	0
35(1)**	10	0	10	11	0	0
35(1)***	9	0	_	8	0	0
35(1)****	5	0	44	_	0	0
35(3)*	19	11	9	22	36	0
35(3)**	9	0	0	11	0	0
35(6)*	14	0	0	19	10	0
35(6)**	0	0	>45	0	0	0
35(7)*	19	0	32	0	10	0
35(7)**	11	0	10	0	0	0
35(7)***	5	0	_	0	0	0
35(7)****	0	0	0	0	0	0
35(8)*	0	0	0	0	0	0
35(8)**	0	0	0	0	0	0
35(10)*	0	0	0	0	9	0
35(10)**	0	0	0	0	9	0

.Key:
* Media of EC = 0.
** Media of EC = 4
*** Media of EC = 8
**** Media of EC = 10
Test pathogens did not grow under such conditions

The overall summary of antibiosis of the *Streptomyces* isolates is presented in Table 12. About 31% of the isolates did not inhibit the plant pathogens tested, but the remaining isolates were inhibitory.

Some isolates produced antimicrobial compounds that inhibited one pathogen, others inhibited two pathogens, and so on. *Clavibacter michiganensis sub sp michiganensis* was the most susceptible pathogen, followed by *Xanthomonas phaseoli, Xanthomonas phaseolicoli* var *fuscoris, Xanthomonas vascatoria* then *Xanthomonas oryzae pv oryzae. Acidovorax avenae* was not affected at all by any of the isolates tested.

The inhibitory effect shown by a majority of the isolates agrees with the observation by Alexander (1983) that the majority of actinomycetes produced antibiotics. This shows some potential of these saline environments to harbour antibiotic producing *Streptomyces*. Therefore this should continue to be explored to get more *Streptomyces* isolates which may have useful applications.

The sensitivity of the pathogens to antibiotics produced by the *Streptomyces* isolates may imply that probably the pathogens had never been exposed to the antibiotics as those produced by the present isolates and therefore, are still susceptible to such compounds. Ndonde (1997) reported a similar phenomenon. Another possibility is that probably the pathogens are simply sensitive to different antibiotics and therefore the antibiotics produced by different *Streptomyces* isolates were not the same.

Those *Streptomyces* which did not show inhibition may be explained in one or two scenarios. One is that they are not antibiotic producers at all.

Ndonde (1998) reported that some *Streptomyces* did not inhibit some plant pathogens. The reasons for failure of *Streptomyces* to produce antibiotics include type of carbon source, nitrogen source, pH and temperature (Dipa, 1977) and salinity (Lee *et al.*, 1997). Lee *et al* (1997) reported that the antibiotic clavanin exerted antimicrobial activity in the presence of 0.1 to 0.3M NaCl concentration, but higher salt concentrations were no antimicrobial activity was noticed. The second scenario is that they may produce antibiotics which could inhibit organisms/bacteria other than those tested presently.

Table 12. Summary of extent of antibiosis by the *Streptomyces isolates*

Extent of inhibition	No of isolates	% of isolates
Strong (>25mm)	11	31.2
Moderate (10 - 24mm)	10	31.2
Weak <10mm	2	6.2
None	10	31.2

4.4.2 Summary of antibiosis of the *Streptomyces* isolates by level of salinity

From Table 13 it can be observed that at least one *Streptomyces* isolate from each level of salinity inhibited at least one plant pathogen. For example, isolates from EC level 0.45 dS/m inhibited two plant pathogens and one isolate from EC level of 7.29 inhibited three pathogens.

This shows that saline environments may also harbour *Streptomyces* which may have some potential of producing antibiotics. Therefore, these environments should be explored further, ecologically as well as *in vitro* influence of salinity on production of antibiotics.

Table 13. Summary of antibiosis of the *Streptomyces* by level of salinity.

Salinity level (dS/m)	Number of isolates tested	Number of *Streptomyces* showing antibiosis	% *Streptomyces* within each salinity level showing antibiosis	Number of pathogens inhibited (out of 6)	% of pathogens inhibited within each salinity level
0.45	7	4	57.1	2	33.3
7.29	1	1	100	3	50
10.9	18	12	66.6	4	66.6
35	6	5	83.3	5	83.3

4.4.3 Summary of antibiosis of the *Streptomyces* isolates by plant pathogen inhibited

Table 14 shows that the tested *Streptomyces* isolates inhibited the plant pathogens, in different proportions. For example, *Xanthomonas phaseoli* was inhibited by 15 *Streptomyces* isolates. *Clavibacter michiganensis sub sp. michiganensis* was inhibited by 16 *Streptomyces* isolates and *Acidovorax avenae* was not inhibited by any of the *Streptomyces* isolates.

Those which produced antimicrobial compounds that affected more than one pathogen probably produced more than one antimicrobial compounds. Brock *et al.* (1994) reported that some *Streptomyces* species can produce more than one antibiotic. Pramer (1996) reported that some *Streptomyces* produced 15 antibiotics. The second explanation is that if the *Streptomyces* isolates each produced only one antibiotic, then it was a broad spectrum antimicrobial compound.

For the case of *Acidovorax avenae* being resistant to antibiotics produced, it may imply that probably the tested *Streptomyces* isolates did not produce antimicrobial compounds which could affect pathogen.

Table 14. Summary of antibiosis by number of pathogens inhibited.

Plant pathogen	Number of *Streptomyces* inhibiting the pathogen	% of the isolates inhibiting the pathogen
Xanthomonas phaseoli	15	45.45
X. oryzae pv oryzae	1	0.03
Clavibacter michiganensis sub sp michiganensis	16	48.5
X. vascatoria	11	33.3
X. phaseolicoli var fuscoris	10	30.3
Acidovorax avenae	0	0

From Table 11, it is clear that most *Streptomyces* isolates inhibited the test pathogens on media with EC zero but they did not do so on media of

EC 4dS/m.

The isolates which produced antibiotics on media of EC zero as well as EC 4dS/m were further tested on media of EC eight and 10dS/m. Pathogens, *X. Phaseolicoli* var *fuscoris* and *X. Oryzae pv oryzae* did not grow on media of EC eight and above. Only *X. Phaseoli* grew on media of EC 10dS/m. Most *Streptomyces* isolates tested under these conditions showed varied degrees of antibiosis e.g. isolates 10.9(6) and 10.9(15). Others did not produce antibiotics at EC 10dS/m, for example an isolate 35(7). This shows that the produced antibiotics are sensitive to salinity in that they are either denatured or inactivated by the presence of salts. This phenomenon has been explained by Jefferys (1952) that some antibiotics can undergo oxidation, reduction, hydrolysis or deamination processes induced, partly, by cations (which contribute to salinity) (Tanji *et al.*, 1967) and, therefore, denaturing the antibiotics. Lee (1997) reported that antibiotics such as clavanin did not show antibiosis on media of 0.3M NaCl concentration or above and, therefore, salinity rendered the antibiotics inactive. Therefore, it is inferred that the antibiotics produced were sensitive to salts. Sensitivity of antibiotics to salinity was also reported by Lee *et al.* (1997) in that at 0.3M NaCl concentration the antibiotic clavanins failed to exert antimicrobial activities.

The second possible explanation is that at that higher salinity level no antibiotic was produced. Since physiological processes of micro-organisms (*Streptomyces* being one of them) are affected by presence high levels of salinity (Band *et al.*, 1992; Pollenko *et al.*, 1981; Killham *et*

al., 1990 and Brown and Salin, 1994) antibiotic production, being a process occurring within the cell, probably was affected by the presence of salts.

4.4.4 Categorization of the *Streptomyces* isolates' extent of antibiosis against the test plant pathogens

From table 15 it is seen that 76.47% of the isolates that showed strong antibiosis against *C. michiganensis* sub sp. michiganensis followed, equally, by *X. phaseoli* and *X. phaseolicoli* var fuscoris which were inhibited by 11.76% of the *Streptomyces* isolates. For the isolates which showed moderate antibiosis, 40% inhibited *C. michiganensis* sub sp michiganensis followed by *X. vascatoria* (35%), *X. phaseolicoli* var fuscoris (20%) then *X. oryzae pv oryzae* (5%). The isolates that showed weak antibiosis had the following trend, 30.76% inhibited *X. phaseoli* followed, equally, by *X. vascatoria.* The isolates were almost equally distributed in terms of their extent of antibiosis, viz., strong, moderate and weak.

The sensitivity of pathogens to antibiotics produced by different *Streptomyces* isolates to different degrees has been discussed in section 4.4.1. The weak antibiosis showed by some isolates may imply that

probably the pathogens were resistant to antibiotics produced by the present *Streptomyces.*

Table 15. Summary of categorisation of the *Streptomyces* isolates' extent of antibiosis against the test plant pathogens

Extent of antibiosis	Plant pathogens inhibited	Number of *Streptomyces* isolates showing antibiosis	% of isolates in each antibiosis category
Strong antibiosis	*X. phaseoli*	2	11.76
	X. oryzae pv oryzae	0	0
	C. michiganensis sub sp. michiganensis	13	76.47
	X. vascatoria	0	
	X. phaseolicoli var fuscoris	2	
	A. avenae	0	11.76
Total		17	99.99
Moderate antibiosis	*X. phaseoli*	0	0
	X. oryzae pv oryzae	1	5
	C. michiganensis sub sp. michiganensis	8	40
	X. vascatoria		
	X. phaseolicoli var fuscoris	7	35
		4	20
	A. avenae	0	0

Total		20	100
Weak antibiosis	*X. phaseoli*	4	30.76
	X. oryzae pv oryzae	1	7.70
	C. michiganensis sub sp. michiganensis	1	7.70
	X. vascatoria	4	30.76
	X. phaseolicoli var fuscoris	3	23.1
	A. avenae	0	0
Total		13	100.02

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY AND CONCLUSIONS

This study was undertaken to isolate and screen for antibiosis of *Streptomyces* form soils of different levels of salinity. Generally *Streptomyces* populations were higher in soils of lower salinity and the vice versa. For example, saline soils with EC level of 35dS/m had populations (log_{10}) of 2.0 per gram of soil and the soils with low EC level of 0.45dS/m had populations (log_{10}) of 4.9 per gram of soil.

There was a significant (P = 0.05) positive correlation between *Streptomyces* populations and total nitrogen. There was a negative correlation between *Streptomyces* populations and salinity levels, meaning that as the level of salinity increased, the population of *Streptomyces* also decreased.

There was no significant relationship (tested by regression analysis) between number of *Streptomyces* and soil characteristics such as organic

carbon, exchangeable bases, pH, or clay content.

Different classes of colours were observed in all locations. The classes of colours of the isolates ranged from grey to white to red types and their intergrades, in different proportions. Different morphological classes were observed in all locations. The morphologies ranged from straight, flexuous to spirals in different proportions. The flexuous morphological type was dominant, followed, equally, by the open loop and the straight types. Based on these morphological criteria, it appears that the assemblage of *Streptomyces* in these saline soils is biodiverse, rather than being dominated by a single isolate or only a few isolates. This has important implications because it indicates that novel strains could be obtained from these samples. These strains may be of interest for screening for novel antibiotics of pharmaceutical importance in addition to antibiotics that inhibit plant pathogens.

All the isolates were able to grow well on all the levels of soil salinity from zero to 35 dS/m . Some isolates grew, to different, on media containing 2%, 5% or 8% salt concentration. None of the isolates grew at 10% salt concentration.

Some isolates inhibited growth of some plant pathogens to different extents. Some isolates showed strong antibiosis, others showed moderate and some showed weak antibiosis while some did not inhibit any of the pathogens tested. The pathogen *Acidovorax avenae* was not inhibited by any of the *Streptomyces* isolates tested. Some isolates (from each level of salinity) produced antimicrobial compounds that inhibited one pathogen others inhibited two pathogens and so on. *Clavibacter michiganensis* sub sp. Michiganensis was the most susceptible pathogen, followed by *Xanthomonas phaseoli*, *Xanthomonas phaseolicoli* var fuscoris, *Xanthomonas vascatoria*, then *Xanthomonas oryzae* pv oryzae. Some pathogens were inhibited by 15 *Streptomyces* isolates, example *Xanthomonas phaseoli* and some were inhibited by 16 *Streptomyces* isolates, for example *Clavibacter michiganensis* sub sp michiganensis.

In conclusion, *Streptomyces* were found in normal soils to soils of high salinity. From this entire salinity gradient, *Streptomyces* were isolated which displayed antimicrobial properties. This may indicate that even extreme environments may harbour *Streptomyces* which may display useful applications like control of plant pathogens.

5.2 RECOMMENDATIONS

In light of results obtained and discussions made, the following recommendations are made:

More isolation be made especially from other areas that are not covered in this study to broaden the *Streptomyces* base

Antibiosis studies be carried out that will include more pathogenic bacteria as well as other groups of pathogens such as fungi and viruses.

Detailed characterisation of the isolated *Streptomyces* using 16S ribosomal RNA sequencing should be attempted to characterise the *Streptomyces* to species level to avoid repetition of studies involving a given species/isolate.

CHAPTER SIX

6.0 REFFERENCES

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