ISOLATION, ENUMERATION AND EVALUATION OF ANTIMICROBIAL POTENTIAL OF *STREPTOMYCES* ISOLATED FROM SOME ACID AND ALKALINE SOILS OF TANZANIA

BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (SOIL SCIENCE AND LAND MANAGEMENT) OF SOKOINE UNIVERSITY OF AGRICULTURE

2000

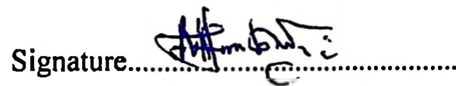
ABSTRACT

The study reported herein was conducted to enumerate and evaluate the antimicrobial potential of *Streptomyces* from acid and alkaline soils. The soils were sampled from Morogoro, Coast and Iringa regions in Tanzania. *Streptomyces* were enumerated using starch-casein agar. Colour of aerial mycelium and morphology of spore chains were determined. The isolates were evaluated for tolerance to pH changes and assessed for antibiosis against selected plant pathogenic bacteria. There were significant (P=0.05) differences in populations of *Streptomyces* in soils of different pH levels. The populations were lowest at pH 4.5 and at pH 10 ($\log_{10}$ values of 3.2 and 3.5, respectively) and were highest at pH 7.2, with a population of 5.4. Regressions analysis revealed that only % clay was significantly (0.05) correlated with *Streptomyces* populations. The regression of *Streptomyces* on all soil pH values was not significant in the entire range. However, the relationship was significant (P=0.05) when *Streptomyces* populations were regressed on pH levels ranging from 4.5 to 7.2. The *Streptomyces* isolates displayed the following morphologies of spore chains: flexuous (60.9%), straight (12.4%), open spirals (13.3%), closed spirals (2.86%), open loops (7.62%), hooks (1.9%) and monoverticillate (0.95%). The colours of mature colonies of *Streptomyces* isolates were white (20%), gray (58.1%), pink (1.9%), red (1.9%), yellow (1.9%) and cream (16.2%). All isolates from acidic soils (pH 4.5 and 5.95) were able to proliferate at high pH up to 7.8 while those from alkalinity conditions (pH 7.85, 8.2 and 10.0) could not do well when tested on the acidic side (pH 4.5 and 5.95).

About 84% of the strains produced antibiotics against *Clavibacter michiganensis* sub sp *michiganensis*, *Xanthomonas phaseoli*, *X. vascatoria*, *X. oryzae* pv oryzae and *Acidovorax avenae*. Six isolates (about 16.2% of all isolates) did not produce antibiosis against any of the tested plant pathogens. *Xanthamonas phaseolicoli* var *fuscoris* was not inhibited by any of the isolates. The *Acidovorax avenae* was inhibited by the least number of isolates, mainly those from acidic soils.

DECLARATION

I, MUSSA FARAJI MVUNGI, do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is a result of my own original work and that it has never been submitted for a degree award in any other University.

Signature..............

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ACKNOWLEDGEMENTS

First of all, I wish to thank God, the Almighty, for his guidance to the success of this work. I am indebted to all individuals who in one way or another facilitated the accomplishment and success of this study. Since it is not possible to mention all of them, I wish to extend to them my sincere acknowledgements.

I wish to gratefully acknowledge the assistance and guidance of my supervisor, Dr. Ernest Semu, who patiently and tirelessly guided me through all the stages of dissertation work. His readiness for help has been the key to the success of this study.

My appreciation also goes to all technical staff in the Soil Science Laboratory of the Sokoine University of Agriculture for their assistance during the laboratory part of this study. Mr. Salum, Mr. Shamte and Mrs Mhaiki deserve special thanks. Friends and colleagues are also acknowledged here for the help I enjoyed in many forms during the course - the school life is enjoyable when you have such good and understanding friends.

Special thanks go to my uncle and my aunt Mr and Mrs Twaha Msuya for their wonderful support during the whole period of my study at the Sokoine University of Agriculture. I wish also to mention the tolerance of my parents who had to suffer inconveniences attributed to the course of this work.

Last but not least, I gratefully acknowledge my employee, La Fleur D'Afrique Limited for granting the permission for further studies. Dr. Milcah Mrema and Mr. Felix Mrema (Hon) deserve special gratitude.

DEDICATION

This work is dedicated to the one who bought me the first pencil I ever used in my life: my mother Hamida.

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CHAPTER ONE

1.0 INTRODUCTION

Attempts to control plant pathogens by use of antibiotics have been made by plant pathologists the world over, aiming at obtaining highly selective control agents that are absorbed by plant or seeds thus enabling the eradication of established infectious even when the pathogen is locked deep inside the plant tissue. These antibiotics might also be of value in cases where the commonly used chemicals may produce injurious side effects on the crops or may be toxic for human beings or animals (Johannes and Dekker, 1963).

Streptomycetes are gram positive bacteria that grow as branching filaments. They form about 75% of all colonies in dilution plates (Alexander, 1961; Lechevalier, 1967). Streptomycetes belong to the broaad group called actinomycetes. Streptomycetes particularly of the genus *Streptomyces* are famous for being prominent producers of antibiotics, that have antibiosis potential to plant and animal diseases, in addition to participating in ecologically important functions like decomposition of organic materials- an important function in soil fertility maintenance.

Soil pH is among the factors affecting *Streptomyces* abundance. Most strains of streptomycetes failed to proliferate or had only negligible activity below pH 5.0. Therefore continuous application of ammonium fertilizers without lime was found to

suppress actinomycetes since ammonia (NH_4^+) is oxidized to NO_3^- by microbial actions and thus resulted in fall in pH which lead to unfavourable growth condition for streptomycetes (Alexander, 1983). Acid tolerant streptomycetes strains were however, encountered and could be demonstrated with ease (Williams *et al.*, 1970). These may extend the useful roles of these organisms to acid soil environments.

The streptomycetes are abundant in relatively high pH soils. Environments with pH less than 5.0 have been shown to affect actinomycetes abundance. Crawford *et al.* (1993) while isolating and characterizing actinomycetes for antagonism to fungal root pathogens found that all 267 strains studied grew well above pH 5.5. He concluded that streptomycetes cannot tolerate acidity. Mitchel (1974) also found that streptomycetes were never found in soils or water below pH 5.0.

The effects of acidity on soil microorganisms may be direct on cell's internal environment or indirect through changes in the external environment and it is seldom possible to distinguish between these. It is generally assumed that of the major microbial groups in the soil, fungi are tolerant to acidity where as most bacteria and actinomycetes are relatively intolerant (Alexander, 1961). In highly acid soils, fungi are regarded as the major component of microbial population.

Reports of adaptations of actinomycetes to acidity or alkalinity are few and Alexander (1961) suggested that scarcity of acid tolerant strains indicated that they had only minor biochemical significance in the soil.

Little evidence of adaptability of streptomycetes to tolerate acidity have already been obtained. Corke and Chase (1964) gave evidence for existence of acid tolerant streptomycetes species.

Some species isolated from podzols were found to be more tolerant to acidic conditions in culture than those from less acid soils. However, these observations were not encountered by many other scientists.

Jensen (1928) isolated a group of streptomycetes which required acidity for growth. He termed the group as *Actinomyces acidophillus*. That group grew in culture only at low pH between 2.6 to 5.5 with no or little growth at pH 6.5. No similar reports of acidophillic actinomycetes have been made, although Corke and Chase (1964) isolated a strain which they considered to be close to *A. acidophillus*.

Also the acidophillic species detected in fresh field soils had similar pH requirements to those of Jen's *A. acidophillus* (Taber, 1960). These microorganisms were probably of considerable importance in the decomposition process in low pH soils. This may be evidence to show that there are possibilities of encountering more streptomycetes species from extreme acid conditions. It was therefore suggested that acidophillic streptomycetes may be more spread and important in the soil than has been thought previously (Williams *et al.*, 1970).

Acid sensitive actinomycetes (alkalophillic actinomycetes) are arbitrarily defined as the ones which require pH 6.0 or more for growth. Few studies on acid sensitive (alkalophillic) actinomycetes have been reported. Actinomycetes grow best in neutral or alkaline media and at one time were thought to require an alkaline reaction for growth (Taber, 1960). The isolation of alkaline dependent *Streptomyces caeruleus* from a region with alkaline soils in Canada, suggested that an acid sensitive actinomycetes flora might exist. This hypothesis was tested by isolating actinomycetes from alkaline and acid soils and comparing their ability to grow at pH 6.2 and 8.5, whereby only 8% of the isolates failed to grow at pH 6.2 (Taber, 1960), implying that *Streptomyces* may tolerate a pH range and not only a discrete value. This raises the need to evaluate this hypothesis to tropical soils.

Alkalophillic actinomycetes can be isolated from the soil using a range of media to pH 10 to 12. A small number of strains classified in the genera *amycolata, saccharothrix* and *Streptomyces* have been proved capable of growth on agar adjusted to pH 11.5 and above (Mikami *et al.*, 1985). However, members of genus *Nicardiopsis* are commonly associated with alkaline conditions and have been isolated from soil using media at pH 11.5 (Mikami *et al.*, 1982; Tsujibo *et al.*, 1988). For some species conditions for growth are above pH 10.0. However, no more reports of alkalophillic actinomycetes have been obtained so far.

Streptomyces are prolific antibiotic producers (Korn-Wendsch and Kutzner, 1992). Most actinomycetes isolated from soils produced antibiotics and were also commonly

associated with the rhizosphere of the plants (Whaley and Boyle, 1967). Many researchers of bacterial pathogens have recorded incidental observations on antibiotic phenomenon in culture or soil. For example, Weindling *et al.* (1950) found that certain micrococci were strongly antagonistic against *Agrobacterium tumefaciens* that cause crown galls in plants. Streptomycin produced by *Steptomyces griseus* showed activity against a broad range of bacterial plant pathogens in-vitro as well as on plant (Johannes and Dekker, 1963). Streptomycetes have been observed in-vitro to produce antibiotics which are capable of inhibiting the growth and development of some fungi, bacteria and nematodes (Alexander, 1983).

Landerkin and Lochhead as quoted by Weindling *et al.* (1950) made a systematic survey of 50 actinomycetes isolated from soil. In general, the strongest antagonists were the most versatile, meaning that they affected the largest number of bacterial pathogens. Streptomycin sprays and dusts are now part of routine diseases control in the U.S. and are officially recommended against fireblight in apples, pears and some ornamental shrubs of the rose family and against walnut blight (Johannes and Dekker, 1963).

It was also observed that, spores of mycelia of the strain *Streptomyces lydicus* were used to coat pear seeds to protect them from invasion by *Pythium ultinum* in oospore enriched soils (Yuan and Crawford, 1995). Some of isolates which inhibited *P. ultinum* were also found to strongly inhibit growth of other root pathogenic fungi. Recent experience suggests that among the metabolites of microorganisms are com-

pounds that will have application on therapeutic agents in a variety of human and animal diseases (Monoghan *et al.*, 1990).

From the observations cited above, the antibiosis potential of *Streptomyces* in inhibiting growth and development of pathogenic microorganisms needs to be studied further. The study of *Streptomyces* with relation to soil characteristics may bring more insight into this potential. If soil conditions of acidity and alkalinity are taken into account, efforts to reveal existence of variety of *Streptomyces* species with antibiosis potential to plant diseases may be more rewarding.

Therefore, because few reports available indicate possibilities of obtaining acid tolerant and acid sensitive strains, there was a need to put more focus on soil conditions of acidity and alkalinity so that important strains of *Streptomyces*, some of which may even have antibiosis potential to plant diseases, may be isolated and studied further.

The present study was an attempt to isolate, enumerate and evaluate the antimicrobial potential strains of *Streptomyces* from soils of acid and alkaline conditions. The specific objectives were:

(i) To enumerate populations of *Streptomyces* from those conditions,

(ii) To compare population of *Streptomyces* from those conditions to normal soils and to evaluate the pH limits of tolerance,

(iii) To relate populations of isolated strains to some other soil characteristics,

(iv) To evaluate *Streptomyces* isolates for their ability to produce antimicrobial compounds that may be of potential in the control of plant diseases.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General aspects of streptomycetes

Streptomycetes are gram positive microorganisms growing as branching filaments. These filaments are segmented in some genera during growth yielding pleomorphic, club-shaped cells that resemble Corynebacterium and Mycobacterium (Davis *et al.*, 1980).

Being transitional between the simple bacteria and the fungi (Alexander, 1983), the actinomycete filaments may sometimes be short, and their individual hyphae appear similar to fungal filaments though they are much smaller, 0.5 to 1.0mm in diameter, analogous to the size of bacterial cells (Krasilnikov, 1958; Mitchel, 1974; Alexander, 1983). Formerly, the actinomycetes were considered as being fungi. However, there are some properties which can group actinomycetes as bacteria. These properties are (Lechevalaer and Lechevalier, 1967):-

(i) They lack nuclear membrane (i.e. they are prokaryotic),

(ii) They have characteristics of bacterial cells such as having cell wall with muramic acid,

(iii) Their growth is inhibited by antibacterial drugs that are innocuous to fungi, e.g. penicillines, tetracyclines, sulfonamides,

(iv) They are insensitive to fungal polyncs due to lack of sterols in cell wall,

(v) Motile forms posses simple flagella of the type found in bacteria,

(vi) Chemoautotrophic and anaerobic forms are known,

(vii) During genetic recombination, one group, streptomycetes, may involve the transfer of a fertility factor and formation of merozygotes instead of the zygote formation seen with fungi.

On the other hand actinomycetes form aerial mycelia and chains of sexual spores similar to those of fungi (Lechevaliar and Solotorovsky, 1965). More than 95% of all actinomycetes in the soil consist of the genus *Streptomyces* (Lechevalaer and Lechevalier, 1967).

2.2 Ecology of *Streptomyces*

Few studies have been done on the ecology of *Streptomyces*. Some studies show that few species of *Streptomyces* can be found in aquatic habitats (Brock *et al.*, 1994). However, *Streptomyces* are mostly soil microorganisms (Brock and Brock, 1973; Mitchel, 1974 and Brock *et al.*, 1994). *Streptomyces* species make up around 50% of the total actinomycete population of the soil, and they grow relatively rapidly and compete succesfully on soil-dilution plates (Goodfellow and Gross, 1984 cited by El-Talabily *et al.*, 1996).

Alkaline and neutral soils are more favourable for the growth of *Streptomyces* than acid soils (Bock *et al.,* 1994). Sandy loams or soils overlying limestone, which are well drained, have been found to contain high numbers of *Streptomyces*. *Streptomyces* are known to require lower water potential for growth than soil simple bacteria (Brock *et al.,* 1994). *Streptomyces* are present not only in surface soil but also in lower soil horizons to a considerable depth. As far as abundance is concerned, they are second to simple bacteria, and viable counts of the two are sometimes almost equal (Alexander, 1983). Although soils are their most important habitat, they can also be found in seawater, cold water, fresh water, guts of warm-blooded animals, and in compost.

2.3 Ecological influences of *Streptomyces* abundance

The number of *Streptomyces* may vary between 1×10^6 and 36×10^6 per gram of soil (Foth, 1984). The number of *Streptomyces* per gram of the soil plough layer (15cm) in humid temperate region was established to be 6×10^{17} which is equivalent to 220kg of its biomass per hectare or 0.01% of the dry soil weight (Foth, 1984).

Streptomyces are found in the A horizon or below the surface. But the cell density declines with depth in the profile in the C horizon, counts may range from 10^3 to 10^5 per gram of soil (Alexander, 1983). Williams and Wellington (1982) reported that their numbers in waterlogged anaerobic soils and in acid soils were often low, at 10^2-10^3 per gram of dry soil.

2.4 Effects of soil pH on abundance of *Streptomyces*

2.4.1 General

Streptomyces are widely distributed in soils, especially in those which are not acid, where propagule numbers are almost equal to the combined counts of all other bacteria (Alexander, 1961). Actinomycetes usually prefer neutral or slightly alkaline soils. Few are able to grow in soils more acid than pH 5.0. However, Williams *et al.* (1970) encountered a group of *Streptomyces* from acid soils that would grow in culture at pH 3.5-5.5. *Streptomyces* may also be common in acid forest litter. Neutrophillic species could only grow in acid soils if the pH value in micro-environments within acid soils had been altered by fungi colonization so that ammonia was released (Williams and Mayfield, 1971). At the other extreme, in alkaline soils (pH 9.0) on Bikini Atoll, 95% of the microflora were actinomycetes (Johnstone, 1947 cited by Williams and Mayfield, 1971). Their predominance probably resulted from their greater resistance to those extreme pH conditions as compared to other organisms.

The highest number of actinomycetes have been reported at soil depth of 11-15cm where pH was almost neutral (Davies and Williams, 1970). The lower numbers reported in shallower parts of soil profiles were due to the limiting effects of the low pH (4.75) in that region (Davies and Williams, 1970). This was in agreement with Jensen (1928; 1930) who isolated few actinomycetes from soils of pH lower than 4.6, there being a gradual increase above that value until maximal numbers were attained between pH 6.8 and 8.0. Similar observations of a preference of actinomycetes for

alkaline soils were noted by Gillespie (1918) as cited by Davies and Williams (1970) and by Corke and Chase (1964).

Corke and Chase (1964) reported the existence of acid tolerant *Streptomyces* species. Some species isolated from podzols were found to be more tolerant to acidic conditions in culture than those from less acid soils. However, many other scientists have not encountered those observations. Jensen (1928) isolated a group of *Streptomyces* which actually required acidity for growth. He termed the group *Actinomyces (Streptomyces) acidophillus* and it grew in culture at pH levels between 2.6 and 5.5. This agrees with Davies and Williams (1970) who reported that lower number of *Streptomyces* populations might be due to the limiting effects of pH (about 4.75).

Williams and Mayfield (1970) found that the number of *Streptomyces* decreased as soil became more acid but was not until pH dropped below 4.2 that they reached zero. They also observed a clear relationship between pH changes induced by amendments and the response of *Streptomyces*. Only those substances which raised pH to around 6.0 stimulated an increase in number of *Streptomyces.* The mechanisms of adaptations of these strains to lower pH soils are not well documented.

Streptomyces numbers are higher in alkaline soils (Alexander, 1983). About 95% of actinonomycetes have been found in certain localities in the Pacific ocean due to the alkalinity prevailing in soils there (Johnson, 1947 cited by Alexander, 1983).

The peats, waterlogged areas and the environments whose pH is less than 5.0, have been found to be unfavourable for streptomycetes (Alexander, 1977; 1983). Crawford *et al.* (1983) isolated 267 strains which grew well at pH levels between 6.5 and 8.0.Very few of those strains failed to grow at pH 6.0, but a significant number failed to grow at pH 5.5. Mitchel (1974) found that *Streptomyces* could not tolerate acidity although, as already mentioned, some *Streptomyces* have still been observed in acid soils (Jensen, 1928; Corke and Chase, 1964; Williams *et al.*, 1970).

2.4.2 Populations of *Streptomyces* in soils of high pH

There are few reports on adaptations of *Streptomyces* to high pH conditions (Alexander, 1961). Davies and Williams (1970) observed that as pH approached 8.0, populations of *Streptomyces* decreased. They recorded populations of 7600 per gram of dry soil at pH 8.0, 2600 colonies per gram of dry soil at pH 8.10, and 1850 colonies at pH 8.60. Ndonde (1998) observed the *Streptomyces* population of 5.19 (expressed as $\log_{10}$) at pH 7.1 with the highest population observed at pH 6.2 which had a mean value of 5.29.

Taber (1960) reported that out of 1269 actinomycetes isolated from 10 alkaline and acid condition soils, 107 isolates were able to grow on glucose asparagine-salt agar which had been adjusted to pH 8.5. Acid sensitive actinomycetes (those requiring a pH of 6.2 or higher) were found in nine out of 10 alkaline soils and in one out of six acid soils (Taber, 1960).

The populations recorded were 332 000 colonies per gram of dry soil at pH 6.2, 2 050 000 colonies per gram of dry soils at pH 7.0, 11 000 000 colonies at pH 7.5 and 228 000 colonies at pH 8.0 (Taber, 1960). When casamino acids were added to soil samples from the C-horizon, with the pH rising to almost 9.0, *Streptomyces* populations decreased (Williams and Mayfield, 1970).

Taber (1960) tested *Streptomyces* for ability to grow at pH 6.2 and 8.5 and found only 8% of the isolates could not grow at pH 6.2. The numbers of actinomycetes antagonistic to *Pythium coloratum*, the causal agent of cavity spot disease of carrots, increased in soils amended with lime (to pH 6.9) (El-Talabily *et al.*, 1996). In similar studies, addition of lime to soils lead to a 100-fold increase in *Streptomyces* populations as compared to unlimed soils (Tsao *et al.*, 1960 as cited by El-Talabily *et al.*, 1996). Tsao *et al.* (1960) found that after addition of lime, soil pH changed to favour growth of actinomycetes which inhibited growth of most fungi.

The factors enabling these organisms to thrive at high pH were reported to include composition of cell membrane lipids, the membrane lipid/protein ratio, very high levels of respiratory chain components in the membrane, a generally more acidic amino acid content in proteins and a Na^+ cycle that facilitates solute uptake and pH homeostasis (Krulwich and Guffanti, 1989). Any or all of these properties could be pre-requisites of an alkalophile. In a study with pH conditional mutants, some non-alkalophillic mutant strains have supported the conclusion that the Na^+/H^+ antiporter, an abundant membrane protein that catalyses rapid protein exchange, that is involved

in pH homeostasis was necessary for growth at high pH (Krulwich and Guffanti, 1989). Extreme alkalophillic actinomycetes and many of alkalophillic *Bacillus* that grow optimally at pH 10.0-11.0 maintain a cytoplasmic pH that is typically two units below the external pH (Krulwich and Guffanti, 1989).

2.4.3 Populations of *Streptomyces* in soils of low pH

Actinomycetes, including *Streptomyces*, are known to be generally intolerant of low pH and the population sizes are adversely related to H^+ ion concentration. Thus, *Streptomyces* prefer neutral to slightly alkaline soils (Brock *et al.*, 1994). Reports of adaptation of actinomycetes to acidic conditions are few (Alexander, 1961). The scarcity of acid tolerant strains may indicate that they have only minor biochemical significance in such soils. In addition, early works have indicated that most soil actinomycetes cease to grow below pH 5.5 (Waksman and Curtis, 1918; Jensen, 1928). Those that grow in very acid soils often comprise less than one percent of the total count (Alexander, 1961).

Davies and Williams (1970) reported the mean number of *Streptomyces* colonies per gram of dry soil of 20 250 at pH 4.75. The low pH was found to be a limiting factor because if pH values approaching neutrality were attained, the population size showed a considerable increase. For example, Davies and Williams (1970) recorded a population increase from 117 500 colonies per gram of dry soil at pH 5.2 to 280 000 at pH 6.90.

In one study, a population of *Streptomyces* of 40 x 10^3 per gram of dry soil at pH 4.4 was observed (Williams and Mayfield, 1970). But when amendments that increased soil pH to 7.0 were added, the population was increased to 80 x10^7 per gram of dry soil (Williams and Mayfield 1970). Williams *et al.* (1970) reported a population of 86 x 10^3 *Streptomyces* per gram in a soil of pH 4.2. However, at pH 7.5 the population observed was 500 x 10^3 as compared to 250 x 10^3 at pH 8.1 (Williams *et al.*, 1970). It was observed that most species originating from acidified media did not grow above pH 6.5 while most of the isolates from neutral media grew below pH 5.5 (Williams *et al.*, 1970). Ndonde (1998) found that the average population of *Streptomyces* at pH 5.8 was 4.6 (expressed as $\log_{10}$).

2.5 Isolation of *Sreptomyces* from soils of different pH conditions

Streptomyces have been isolated from soils of different pH conditions. More *Streptomyces* strains have been observed in soils of pH 6.5 to 7.5 when compared to acid soils or more alkaline conditions. Isolation of alkaline dependent *Streptomyces caerulens* from a region with alkaline soils in Canada suggested that an acid sensitive actinomycete flora might exist (Taber, 1960). Alkalophillic actnomycete can be isolated from the alkaline soils using media with pH adjusted to 10 and 12. Some strains of *Streptomyces* have been isolated and proved to be capable of growing on agar media adjusted to pH 11.5 or above (Mikami *et al.*, 1985). For some species favourable conditions for isolation and growth were at pH 10.0 or above.

Strains of *Streptomyces* have also been isolated (Harikoshi and Akiba, 1982 cited by Mikami *et al.*, 1985) that can grow in alkaline media of pH 10 to 11.

Most microorganisms were found in less extreme environments and the vast majority of all those species grew best in the neutral pH range; these were referred to as neutrophilles (Krulwich and Guffanti, 1989). There was, however, a diverse group of bacteria that thrived in highly alkaline environments. Those were separated into two broad categories, alkali-tolerant organisms, which showed optimal growth in the pH range of 7-9 but could not grow above pH 9.5. The second category was the alkalophillic organisms which showed optimal growth above pH 9.5 and others which showed optimal growth between pH 10.0 or 12.0 (Krulwich and Guffanti,1989).

Results from testing of organisms originally isolated from acidified and from neutral pH media showed that these groups had different pH requirements (Williams *et al.*, 1970). Most species originating from acidified media did not grow above pH 6.5, while most of isolates from neutral media grew below pH 5.5 (Williams *et al.*, 1970). In another study, none of the *Streptomyces* originally isolated from pH 4.5 were able to grow at pH 2.5, while eight isolates grew at pH 5.5, six at pH 6.5, four at pH 7.3, and only one isolate at pH 8.5 (Williams *et al.*, 1970).

On the other hand, the isolates from isolation medium of pH 7.0 were able to grow at different pH values, with the number of isolates able to grow in each test pH level shown in bracket as follows: pH 2.5(0), pH 3.5(1), pH 4.5(1), pH 5.5(4), pH 4.75(4)

and pH 8.5(3) (Williams *et al.*, 1970). Those isolates which grew in culture at pH the range of 3.5 to 6.5 were termed acidophillic and those grew in culture at pH 5.5 to 8.5 were termed neutrophillic (Williams *et al.*, 1970).

The general pattern which emerges here is that there is a need to adjust conditions of culture media to simulate the original environment in order to capture *Streptomyces* from respective pH conditions.

2.6 Effects of soil pH on metabolic activities of *Streptomyces*

Many metabolic activities of *Streptomyces* have been reported to be affected by pH changes. For example, some *Streptomyces* have been reported to produce glutamate dehydrogenase, an enzyme which is responsible for ammonia assimilation and glutamate catabolism in organisms. Its action was found to be optimal between pH 8.2 and 9.2 (Millard and Taylor, 1927 cited by Taber, 1960). Most strains of *Streptomyces* and related forms failed to proliferate, or had negligible activity, below pH 5.0 or less, thus precluding high populations in such soils (Taber, 1960). This may be due to suppression of some enzyme systems at the lower pH conditions.

Addition of chitin to soils has been shown to stimulate actinomycetes and suppress development of certain plant pathogenic fungi (Mitchell and Alexander, 1962; Mitchell, 1963 cited by Davies and Williams, 1970). There is no doubt that many *Streptomyces* can produce chitinase enzymes (Jeauniaux, 1955, Lingappa and Lock-

wood, 1961; 1962 as cited by Davies and Williams, 1970) and that chitin addition provides them with readily available source of nutrients that eventually increase their metabolic activities. The chitin action was very pronounced when, among other things, pH was adjusted to 7.0 (Reynolds 1954).

The proteolytic activity on casein of protease enzymes from *Streptomyces corchorussii* isolated from soils of Egypt was found to be maximal at around pH 11, although the enzyme showed activity at pH 5.0 and 12 (El-Shanshoury *et al.*, 1994). In acid soils considerable amounts of fungal mycelia, arthropod exoskeletons and other organic nitrogenous material occur (Williams and Mayfield, 1970). As these were decomposed by acid tolerant organisms or by autolysis, they released ammonia (Williams and Mayfield, 1970).

A resting *Streptomyces* conidium in such a site where ammonia is produced could then germinate and produce a mycelium and more conidia. Those newly formed conidia could remain dormant in acid soils until they die or come into contact with another suitable microsite (with higher pH due to ammonia) (Williams and Mayfield, 1970). The occurrence of such restricted microsites was also suggested, by presence of small numbers of nitrifying bacteria requiring both ammonia and higher pH for activity, in acid forest soils with pH of 3.7-3.9 (Chase *et al.*, 1962, as cited by Williams and Mayfield, 1970).

Low pH might prevent microbial growth by one of several mechanisms. The fact that at low pH *Streptomyces* innocula can only expand slightly but without leading to continued growth suggested that while some nutrients were accessible to cells, others were not and thus after exhaustion of reserves, growth of *Streptomyces* ceased (Taber, 1960). The effects of pH on nutrient access to cells are not well documented. However, it is possible that pH affects the accessibility of nutrients to cells in the same manner as it does for availability of nutrients to plants whereby low pH results in fixation of some elements like phosphorus, and also makes some micro-nutrients unavailable to plants.

Assimilation of glucose, which was prevented by low pH, was thought to lead the inability of cells to respire exogenous glucose (Taber, 1960). Taber (1960) observed that all isolates under study were able to oxidize exogenous glucose at pH 6.2. This increased overall respiration, which eventually may have contributed to increased *Streptomyces* activities, growth and populations.

2.7 Effects of pH on production and stability of antibiotics produced by *Streptomyces*

Few reports are available on favourable pH ranges for antibiotic productions by *Streptomyces* and on stability of antibiotics. Antibiotics have been reported to be produced at pH ranges that are also favourable for *Streptomyces* growth.

In aquous solutions antibiotics were found to be unstable, more so as the pH approached 7.0 (Hays *et al.,* 1948). Hays *et al.* (1948) also reported that pH 6.8 was optimum for growth of *Penicillium* while pH 7.3 was best for penicillin formation. Application of lime (to pH 6.9) was found to reduce cavity spot incidence in carrots (El-Talabily *et al.,* 1996) by increasing antibiotic production as pH was raised.

The rate of destruction of antibiotics in soil varies. Some of them were inactivated within a few hours and others were stable for a few days or even weeks, depending on the nature and the properties of the substance (Krasil'nikov, 1958). Antibiotics were found to vary greatly on stability and this depends first of all, upon the properties of substances, and secondly, on the type of soil and external conditions like pH and are most rapidly inactivated in krasnozems and podsols soils (Krasil'nikov, 1958).

Antibiotics with basic properties, for example streptomycin, were quickly inactivated in the soil. Neutral antibiotics, for example chloromycetin, were inactivated slowly, and stability of antibiotics of the acidic type was intermediate (Krasil'nikov, 1958). Adsorption, with subsequent inactivation, of antibiotic substances depended to a considerable degree on soil acidity (Krasil'nikov, 1958). At pH 3.2, soils rich in humus absorbed 4000μg of antibiotics per gram and only 400μg/g, i.e. ten times less at pH 5.6-7.6 (Krasil'nikov, 1958). According to Gregory *et al.* (1952) cited by Krasil'nikov (1958), the antibiotic actidione was stable in alkaline soils at pH 7.8 for eight days and in acid soils at pH 5.2 for more than 14 days.

Consequently, the antimicrobial action of antibiotics differed in different soils. In order to inhibit growth of *Bacillus polymyxa* in soil at pH 5.6 a concentration of 5,000µg/g of terramycin in soil was required (Gottieb and Siminoff, 1952 cited by Krasil'nikov, 1958). Stability of antibiotics in the soil was also found to vary with the latter. Control of crown galls (*Agrobacteria tumefaciens*) in roses at La Fleur D'Afrique farm in Arusha Region, Tanzania has been more effective when the antibiotics like *Streptomycin sulphate* was mixed with water and the solution buffered at pH 6.5-7.0 and then sprayed onto the galls. This has been observed to make the antibiotic stable and effective against the galls. However, when the antibiotics was sprayed on the galls using solution of pH below 6.5 or above 7.0 the effectiveness and stability was reduced (Wright, 1998, personal communication).

From the above review, it appears that the production, stability and effectiveness of most antibiotics produced was obtained at the pH ranging from 5.2 to 8.0, depending on the nature of antibiotics.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 Locations of soil collection

Soil samples were collected from different areas around Morogoro which had different pH conditions. These included Mkata (along the road to Mkata ranch), Maharaka, SUA-TU plot (near Maharaka Primary School some 10km off Doma along the Morogoro-Iringa road), Kikundi (near Kikundi Primary School within Morogoro town), SUA Farm at Magadu (at the Rock phosphate trial site). Another area included Mafiga, along the Morogoro-Iringa road. Some locations of Coast region sampled were the Ruvu NAFCO farm, Chalinze Mzee (several km from Chalinze along the Chalinze-Segera road) and Kibena tea farm of the Tanganyika Wattle Company in Njombe in Iringa region.

3.2 Soil sampling and processing

Representative soil samples within an area of virgin land were collected to a depth of 10cm. One kg composite samples were collected and taken to the laboratory where they were subdivided into two almost equal parts. One part was stored in a cool, dry place for up to two weeks for microbiological studies while the other was air-dried, ground and passed through a 2mm sieve for analysis of chemical and physical properties.

3.3 Soil analysis

3.3.1 Particle size analysis

Particle size analysis was determined using the Bouyoucos hydrometer method as described by the National Soil Service (1990). Fifty grammes of air-dried soil were weighed into 250ml polythene bottles and mixed with 50ml of the dispensing agent (5% sodium hexametaphosphate solution). Distilled water was added to make about 200ml and the suspension shaken and left to stand overnight. The samples were then shaken for two hours, with the bottles held in horizontal position on a reciprocating shaker, at 150 revolutions per minute (r.p.m). The suspensions were quantitatively transferred to one litre sedimentation cylinders and the volume of mixture made to one litre with distilled water. Hydrometer readings were taken after 40 seconds, 4 minutes and 2 hours after which the relative size (i.e. % sand, % silt, and % clay) proportions were calculated. From these data, the soil's textures were determined using the USDA textural triangle.

3.3.2 Soil pH

The soil pH was determined in water (at the soil: water ratio of 1:2.5). A 10g soil sample was placed in a 100ml beaker and 25ml of distilled water were added. The suspension was shaken using mechanical shaker for 30 minutes, left to stand for a few minutes and pH measured using the glass electrode.

3.3.3 Organic carbon

The organic carbon content of the soils was determined by the Walkley-Black method (Nelson and Sommers, 1982). One gramme soil was weighed in a 250ml conical flask and mixed with 10ml 1N $K_2Cr_2O_7$ and 20ml concentrated H_2SO_4 to oxidize the organic carbon. Ten ml of concentrated phosphoric acid were added and the flasks allowed to stand for 30 minutes after which 200ml of distilled water were added to stop the reaction. One ml diphenylamine indicator solution was added and excess dichromate was titrated with 0.5M ferrous ammonium sulphate to the end point (dark green). The amount of dichromate reduced was used to calculate the organic carbon content in the soil.

3.3.4 Total nitrogen

Total nitrogen was determined by the micro-Kjeldahl method (National Soil Service, 1990). One gramme of soil was digested in a Kjeldahl flask for 3 hours. Then 75ml of distilled water were added and the digest left to cool. It was distilled in the presence of 40% NaOH, with the distillate collected into 5% boric acid solution.

The distillation was continued until 200ml were collected. A blank (no soil but reagents only) was similarly processed. Titration of soil samples and blank was done with 0.05M sulphuric acid to the end point (colour change from green to light red). The total nitrogen was calculated from the titration readings (National Soil Service, 1990).

3.3.5 Extractable phosphorus

The Bray and Kurtz 1 method (Page *et al.*, 1982) and the Olsen's method (Murphy and Riley, 1962; Watanabe and Olsen, 1965) were used to determine the extractable phosphorus. The Bray method was used for the soils from Kibena tea farm, SUA farm (two sites), Ruvu NAFCO area, whose pH were below 7. For those from Mkata ranch, Maharaka and Kikundi, where the pH was above 7 the Olsen method was used. In the case of the Bray method, five grammes of soil were weighed into a 50ml plastic bottle and 35ml of the Bray 1 extracting solution (dilute HCl -NH_4F) were added and the bottles shaken as required. They were filtered by a Whatman No. 40 filter paper.

Five ml of the filtrate were pipetted into a 50ml volumetric flask for 15 15 minutes and 15ml distilled water added. Ten ml of 1.65% boric acid was added into the volumetric flask and mixed. Ten ml of the colour-forming reagent (ascorbic acid-molybdate blue) were added into the volumetric flask and the contents made to volume using distilled water and thoroughly mixed. They were left to stand for 30 minutes for the blue colour to develop. Absorbance was measured in a 10 mm cuvette at 882nm with appropriate standards included. The Bray extractable phosphorus content of the soils was calculated.

For Olsen's method, 5 grammes of soil were weighed into a 250 ml plastic bottle. One hundred ml 0.5 M $NaHCO_3$ were added. The bottles were shaken for 30 minutes on a mechanical shaker at 160 r.p.m. and filtered. Five ml of the filtrate were pipetted

and transferred to a 50 ml volumetric flask. Then 0.3M sulfuric acid was added till a strong effervescence occurred. The flask was intermittently shaken for 30 minutes to complete effervescence after which 30 ml water were added, and shaken to mix. Thereafter, 10ml of ammonium molybdate-ascorbic acid solution were added, made to volume with distilled water and mixed for colour development. After 15 minutes, absorbance was measured on a spectrophotometer at 890nm, and Olsen extractable phosphorus calculated.

3.3.6 Cation exchange capacity

Five grammes of soil were mixed with 1M neutral NH_4OAc solution (pH 7) (National Soil Service, 1990). The suspension was stirred, filtered into a 100ml volumetric flask and the residual soil on the filter paper washed with NH_4OAc solution until the volume of the filtrate just approached the 100ml mark of the volumetric flask. Ammonium acetate was used to fill the flask to the mark.

The residual soil was washed in the filter paper using 100ml of 80% ethanol, after which the soil on filter paper was suspended in acidified 1M KCl solution, and filtered. Twenty ml of the KCl filtrate was pippeted into distillation tubes, mixed with 40% NaOH and distilled. The distillate was collected into boric acid, titrated with 0.05N H_2S0_4 as for total N, the CEC calculated.

3.3.7 Brief description of the properties of the soils used

The properties of the soils are given in Table 1. The pH of the soils varied widely, as was required, from 4.5 to 10.1. The Kibena (Njombe) tea growing soil had the lowest pH value and the Kikundi (Morogoro) soil had the highest. Thus, both Kibena and SUA farm (Magadu) soils were strongly acidic while the Ruvu Soil (Ruvu NAFCO farm), other SUA soils and the Mkata soil were neutral to slightly acidic conditions. The remaining soils were on the alkaline range.

Table 1. Some properties of the soils used

Site	pH(1:2.5) H_2O	%OC	%Total N	Extractable P, ppm	%Sand	%Silt	%Clay	Textural Class
Kibena tea project	4.5	1.47	0.08	2.03	60	2	38	Sand clay
SUA farm (Magadu)	5.2	1.04	0.03	0.68	41	6	53	Clay
Ruvu NAFCO area	5.95	1.33	0.03	0.18	11	18	71	Clay
SUA farm, Mafiga	6.65	1.72	0.03	0.25	31	12	57	Clay
Mkata, road side	7.2	1.60	0.02	3.32	9	20	71	Clay
Maharaka	7.85	1.07	0.02	1.47	55	14	31	Sand clay
Mkata, off road	8.27	1.13	0.01	0.29	13	18	69	Clay
Kikundi	10.1	1.84	0.02	0.22	11	18	71	Clay loam

The organic carbon content of the soils ranged from 1.04% to 1.8%, which may be rated as low. Total nitrogen was generally low, ranging from 0.01% to 0.08%. The extractable phosphorus was generally low, the highest value being 3.3mg/kg. Clay content ranged from 31% (Maharaka) to 71% (Mkata) making the textures of all the soils to vary from sandy clay to clay.

3.4 Enumeration of *Streptomyces*

The enumeration of *Streptomyces* was done using the plate count method with starch-casein agar medium (Kuster and Williams, 1964; Williams and Wellington, 1982; Wollum, 1982). Before making the media for the isolation of *Streptomyces*, the buffering solution of KH_2PO_4 (pH 4.5) and K_2HPO_4 (pH 9.2) were prepared and used in adjusting the media at different pH levels. The medium was prepared and pH adjusted to simulate the pH of the collected soils with values ranging from 4.5 to 10.1 using the buffering solutions made earlier. The pH values of different medium was obtained by randomly mixing the two solutions until the required pH value was obtained.

The media were sterilized in an autoclave at 15 pounds per square inch and 121°C for 15 minutes. The media were also fortified by adding (aseptically) Penicillin G and actidione (cycloheximide) before pouring into petridishes to suppress the growth of bacteria and fungi, respectively (Williams and Davies, 1964; Porter *et al.*, 1960). Petridishes and pipettes were sterilized in oven at 170°C for two hours prior to use.

Ten grammes of soil (oven dry equivalent) were placed, in four replicates, into bottles containing 90ml of sterilized distilled water and shaken thoroughly to detach microbial cells from the soil particles into the soil suspension. This was the 10^1 suspension of the soil. Then, ten-fold serial dilutions were made to get dilution required for plating. One ml portions from three dilution levels i.e. 10^3, 10^4 and 10^5 were plated in duplicate and incubated at 25°C to 28°C for two weeks after which *Streptomyces* colonies were counted from dilution levels showing a good distribution of colonies.

Counting was initially done on 14th day and the plates were allowed to stay in the incubator for seven more days to give more time for better growth of *Streptomyces* in the plates with extreme pH conditions. After that period re-counting of all plates were done.

The colony counts obtained were converted to *Streptomyces* population per gram of oven dry soil after which the data were transformed to the logarithmic (base10) scale. The transformed data were subjected to analysis of variance, using the completely randomized design, to evaluate the effects of location and pH changes on *Streptomyces* populations. The data were also regressed on soil pH, organic carbon, total nitrogen, extractable phosphorus, clay and CEC to assess the influence of these parameters on the populations.

3.5 Isolation of *Streptomyces*

The lamina flow chamber was used during the isolation of *Streptomyces*. The chamber was first sterilized using U.V. radiation from an in-built ultraviolet lamp for 30 minutes. Portions of *Streptomyces* colonies from starch casein agar plates from section 3.4 were picked using a sterile inoculating loop and transferred to plates with oatmeal agar (Kuster and Williams 1964). The oatmeal agar was prepared to the different pH 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 section 3.6 to 3.9 below.

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

The colour of aerial mature sporulating mycelium was determined after transferring *Streptomyces* colonies from each pH level from starch casein agar to oatmeal agar medium (Kuster, 1959) adjusted to the respective pH value. The colour was determined by assigning colour names given by Pridham (1965) due to unavailability of better facilities.

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3.7 Determination of morphology of the isolates' spore chains

A small peripheral portion of a colony well grown and mature part of a colony was picked using a sterile inoculating loop and transferred to a microscope slide. Morphology of spore chains was observed under a (Nikkon 98455) light microscope (Shirling and Gotieb, 1966) at the magnification of 600x. The observed morphology was compared and assigned to one of the classes described by Pridham *et al.* (1958), Shirling and Gottieb (1966) and Brock *et al.* (1994). Several fields were examined to confirm a morphological class.

3.8 Evaluating the range of pH tolerance of *Streptomyces* isolates

The range of tolerance of different *Streptomyces* isolates was determined using oatmeal agar adjusted to the pH of the soil samples using the buffers prepared from solutions of KH_2PO_4 and K_2HPO_4. The *Streptomyces* isolated from various pH levels were tested for the range of pH tolerance in culture as follows:-

The *Streptomyces* originally isolated from pH 4.5 were tested at pH 4.0, 5.0, 7.0 and 9.0. Those isolated at pH 5.95 were tested their limits of tolerance at pH 4.0, 7.0 and 9.0. The *Streptomyces* from pH 6.65 were tested at pH 4.0, 5.0, 9.0 and 10.0 and those from pH 7.2 were tested at pH 4.0, 5.0, 8.0 and 9.0. The ones isolated from pH 7.85 were tested at pH 4.0, 5.0 and 9.0 while those from pH 8.2 were tested at pH 4.0, 6.0, 9.0 and 10.0. The limits of tolerance of *Streptomyces* isolated from pH 10.1 were studied at pH 4.0, 5.0, 7.0, 8.0 and 9.0.

The isolates were incubated at 25-28°C for 14 days (Harikoshi, 1971) after which the range of the pH tolerance of the different isolates were established by examining growth at the different pH levels tested.

3.9 Determining the ability of *Streptomyces* isolates to inhibit growth of bacterial plant pathogens at different pH levels

The isolates of *Streptomyces* were transferred from oatmeal agar plates to nutrient agar plates using an inoculating loop. A straight line of *Streptomyces* innoculum was streaked across the nutrient agar medium in a petridish. The inoculated plates were incubated for 3 days after which the test microorganisms were streaked at right angles to the *Streptomyces* line as described by Prescott and Dunn (1959), Alexander (1983) and Brock *et al.* (1994). The test organisms were plant pathogens *Acidovorax avenae*, *Clavibacter michiganensis sub sp michiganensis*, *Xanthomonas oryzae* pv oryzae, *X. vascatoria*, *X. phaseolicoili* var fuscoris and *X. phaseoli.* Some characteristics of these pathogens are presented in Table 2. These test bacteria were first cultured in nutrient broth at 25^{0}C for 3 days to obtain the source of innoculum. The pH levels of the media used for testing antibiosis were selected on the basis of pH levels at which the *Streptomyces* were still able to grow. The selected pH level were those whereby *Streptomyces* isolates grew well when the ranges of tolerance to pH were evaluated in section 3.8.

However, before introducing the test organisms, each of these bacteria was grown in nutrient agar media at different pH levels to examine their ability to grow at those different pH values. Most of the test bacteria (except *Acidovorax avenae*) were able to grow at all the pH levels.

After streaking the test organisms at right angle, the plates were incubated for a further three to seven days. The extent of inhibition of the growth of the pathogens by *Streptomyces* was recorded by measuring the length of inhibited zone, in millimeters (mm).

Table 2. Some characteristics/features of the test bacteria used to evaluate antibiosis by *Streptomyces* isolates

Organism	Gram reaction	Significance/importance
Acidovorax avenae	-	Cause bacterial leaf of rice
Clavibacter michiganensis sub sp *michiganensis*	-	Causes bacterial canker of tomato
Xanthomonas oryzae pv oryzae	-	Causes bacterial blight of 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 Population of *Streptomyces* in soils of different pH conditions

Table 3 shows the populations of *Streptomyces* (expressed as log 10). There were significant differences (P=0.05) in *Streptomyces* populations between different pH levels which simulated pH conditions of the different soils. The *Streptomyces* populations were low at pH 4.5 and at pH 10, and were highest at pH 7.2.

The lower populations obtained at pH 4.5 agree with observations by Crawford *et al.* (1993) who found that environments with pH less than 5.0 affected the abundance of actinomycetes. Similarly Jensen (1928) found few strains of *Streptomyces* capable of proliferating at pH 2.5-5.5. Corke and Chase (1964) also found little evidence of existence of acid tolerant *Streptomyces*.

A possible explanation to the low *Streptomyces* populations in very acid soils may be that most enzyme systems become inactivated under very acid conditions. In this way, the cell fails to carry some of its metabolic functions, fails to grow and proliferate, and, eventually dies. Therefore, populations remain low. An example of mechanism which prevents the growth of *Streptomyces* at low pH is that, at this condition the continued growth of the introduced innoculum fails.

Table 3. Population of *Streptomyces* as obtained from different soil pH levels

Location	Soil pH	Population
		$\log_{10}$
Kibena tea farm, Njombe	4.5	3.2c ± 0.3
SUA farm (Magadu)	5.2	3.5c ± 0.2
Ruvu NAFCO farm	5.95	3.7c ± 0.2
SUA farm (Mafiga)	6.65	4.4b ± 1.0
Maharaka	7.2	5.4a ± 0.3
Mkata bridge (road side)	7.85	4.4b ± 0.2
Mkata bridge (off- road)	8.2	3.9c ± 0.1
Kikundi area	10.1	3.5c ± 0.4

Streptomyces population means within a column followed by the same letter were not significantly (P=0.05) different according to the Duncan's New Multiple Range Test.

This may suggest that while some nutrients or metabolites were accessible to cells, others were not and thus after exhaustion of reserves growth ceases. Assimilation of glucose was prevented by low pH, and this effect might be reflected in the inability of cells to respire exogenous glucose (Taber, 1960).

The few reports of existence of acid tolerant *Streptomyces* strains in low pH soils (e.g. pH of 4.5) implies that some *Streptomyces* variants may exist whose metabolic systems have successfully adjusted themselves to such acidic conditions. The next question remains as to whether such strains can carry out their functions efficiently under those conditions or whether the tolerance is just a survival mechanism until favourable conditions prevail again (e.g. if a spore is blown by winds and falls on a more conducive soil elsewhere). An argument to this effect is presented in section 4.6 where for some isolates antibiosis increased when *Streptomyces* strains from relatively more acidic conditions were cultured under conditions of higher pH.

The lower populations recorded at the higher pH conditions may also be explained in terms of impaired metabolic functions at very high pH levels. For example, cellulases produced by fungi and simple bacteria were found to be active over a pH range 4 to 6, but inactive at alkaline pH values (Fumiyasu *et al.*, 1985). This may happen with *Streptomyces* and may ultimately lead to low populations under high pH levels as cell proliferation becomes impaired.

The higher populations at about neutrality (pH 7.2 in the present studies) is rationalized in terms of the ideal conditions prevailing, which are conducive to high rates of metabolism and multiplication. Similarly, Davies and Williams (1970) observed high *Streptomyces* populations in soils whose pH was at neutrality and low *Streptomyces* populations in low pH soils (pH of about 4.75).

The low *Streptomyces* populations under very extreme conditions of acidity or alkalinity (pH 4.5, or 8.2 and 10.1) were observed when culture media were buffered at the respective pH values to simulate the pH conditions of the original soils. These population levels may reflect the natural *Streptomyces* populations in those soils at their respective pH values. The occurrence of *Streptomyces* in soils of extreme conditions of acidity or alkalinity needs to be studied further to explore possibilities of occurrence of economically important strains.

4.2 Relationships between *Streptomyces* populations and some soil characteristics

Table 4 shows the results of regression of *Streptomyces* populations on different soil characteristics. With exception of clay content, the characteristics of the soils used in this study had no great influence on *Streptomyces* populations. The overall regression of *Streptomyces* populations on soil pH was found to be not significant (P=0.05). However, the relationship was significant when *Streptomyces* populations were regressed on pH levels ranging from 4.5 to 7.2. There was no significant relationship

Table 4. Regressions of *Streptomyces* population on different soil parameters

Parameter	R^2	P value
Total N	0.0096	0.82 NS
CEC	0.0554	0.57NS
Extractable P (from pH 4.5 to 10.1)	0.0291	0.68NS
Extractable P (from pH 4.5 to 7.2)	0.4771	0.20NS
Extractable P (from pH 7.2 to 10.1)	0.6433	0.02*
% 0C	0.0001	0.82NS
Clay	0.5455	0.03*
Silt	0.0097	0.98NS
Sand	0.0350	0.12NS
PH (from pH 4.5 to 10.1)	0.0489	0.59NS
PH (from 4.5 to 7.2)	0.8774	0.02*
PH (from 7.2-10.1)	0.7343	0.14NS

NS= Not significant

when the *Streptomyces* populations were regressed on pH ranges from 7.2 to 10.0. When the same approach was used for % silt, % clay and extractable P, only extractable P was found to be significantly (P=0.02) related to *Streptomyces* populations in the pH range from 7.2 to 10.1.The significant (P=0.05) regression of *Streptomyces* population on % clay may be attributed to the relatively fine texture of the soils, which may have adsorbed *Streptomyces* and contributed to the higher population levels observed.

The significant regression of *Streptomyces* populations in soils on pH range 4.5-7.2 may be related to the conditions favourable for *Streptomyces* growth. The low populations at pH 4.5 may be attributed to the prevailing high H^+ ion concentrations. The low pH might prevent growth of more *Streptomyces* by several mechanisms. The rise in pH from 4.5 to 7.2 imparts good conditions conducive to high metabolic rates. Low pH might have prevented assimilation of glucose, leading to inability of cells to respire exogenous glucose as was observed by Taber (1960). As pH increased to 7.2 the ability of cells to respire exogenous glucose might have increased and this may have contributed to higher rates of cell growth and multiplication, thereby increasing the *Streptomyces* populations. Taber (1960) identified *Streptomyces* isolates which were able to respire more glucose at higher pH.

The significant regression of *Streptomyces* populations on extractable P in soils of pH ranging from 7.2 to 10.1 may be due to different reasons. *Streptomyces* populations being higher at pH 7.2 and low at pH 10.1 may be related to extractable P. Be-

cause soils with pH 7.2 had high extractable P as compared to soils of pH 10.1 (Table 1), this might have contributed to higher *Streptomyces* populations in that pH range.

4.3 Preliminary characterization of *Streptomyces* isolates according to colour of aerial mycelium

Table 5 gives the range of colours of the aerial mycelia of some *Streptomyces* isolated from each soil as a measure of distribution and variability. The classes of colours of the isolates ranged from gray to white to cream to yellow, red and pink types and their intergrades, in different proportions. The gray series was dominant. The occurrence/repetition of different colours is summarized and presented in Tables 6 and Table 7. Table 6 shows the overall dominance of the gray class followed by the white and cream classes, in that order. Other workers, as summarized by Pridham (1964) reported similar observations. The dominance of the gray series was also observed by Ndonde (1998) using soils from other locations in Tanzania. Although gray series was generally dominant, the white series were found to dominate within each pH level, followed by gray series (Table 7).

The different categories of colour observed signify a wide ranges of variability in the isolates. However, because methods of colour determination of *Streptomyces* have not been standardized internationally (Pridham, 1965), colour variability cannot be used as criterion in designating species. The dominance of the white series within each pH level (Table 7) implies that the overall dominant colour of aerial mycelium

may not necessarily be the one dominating at every individual pH. For example, the gray series were not encountered at pH 5.95 (Table 7). This may imply that such a series is sensitive to the conditions imposed by this pH and, therefore, could not grow well under that pH.

Table 5. Colour of aerial mycelium of *Streptomyces* isolates as observed on oat meal agar

pH(+ Strain no.)	Colour
4.5(1)	White
4.5(2)	White
4.5(3)	White
4.5(6)	White
4.5(41)	Gray
5 95(16)	White
5 95(7)	White
5.95(39)	Whitish
5.95(34)	Light yellow
5 95(10)	Yellow
5.95(11)	Cream
6.65(16)	Light yellow
6.65(13)	Gray white
6.65(19)	Gray
7.2(14)	White
7 2(15)	White
7.2(16)	White
7.2(17)	White
7.2(18)	White
7.2(19)	White
7 2(20)	White
7.2(21)	White
7.2(22)	White
7.2(23)	White
7.2(24)	Cream
7.2(25)	Cream
7.2(35)	Cream
7.2(27)	Cream
7.2(12)	Gray
7.2(29)	Gray
7 2(4)	Grayish
7.2(11)	Whitish
7.2(32)	Gray
7.2(33)	Gray
7.2(34)	Gray
7.2(35)	Gray
7.2(36)	Gray
7 2(37)	White gray
7.2(38)	White gray
7.2(39)	White gray
7.2(40)	White gray
7.2(41)	White gray
7.2(42)	White gray
7.2(43)	White gray
7.2(44)	White gray
7.2(45)	White gray
7.2(5)	Light gray
8.2(28)	White
8.2(58)	Gray
8.2(48)	White
8.2(1)	White gray
8.2(2)	White gray
8.2(3)	White gray
8.2(4)	White gray
8.2(5)	White gray
8.2(6)	White gray
8.2(7)	White gray
8.2(8)	White gray

8.2(9)	White gray
8.2(10)	White gray
8.2(11)	White gray
8.2(12)	White gray
8.2(13)	Red
8.2(14)	Reddish
8.2(15)	White gray
8.2(16)	White gray
8.2(49)	Yellow pink
8.2(50)	Gray
8.2(38)	Gray
8.2(18)	Light gray
8.2(19)	Light gray
8.2(20)	Light gray
8.2(21)	Light gray
8.2(22)	Light gray
8.2(23)	Light gray
8.2(24)	Light gray
8.2(25)	Light gray
8.2(26)	Light gray
8.2(27)	Light gray
8.2(28)	Light gray
8.2(29)	Pink
8.2(30)	Light gray
8.2(31)	Light gray
8.2(32)	Light gray
8.2(33)	Light gray
8.2(34)	Cream
8.2(35)	Cream
8.2(36)	Cream
8.2(37)	Cream
8.2(38)	Cream
8.2(39)	Cream
8.2(40)	Cream
10.1(16Q)	White gray
10.1(17Q)	White gray
10.1(18Q)	White gray
10.1(19Q)	White gray
10.1(20Q)	White gray
10.1(21Q)	Milk white
10.1(44)	White gray
10.1(10C1)	Cream
10.1(10C2)	Cream
10.1(10C3)	Cream
10.1(10C4)	Cream
10.1(10C5)	Cream
10.1(10C6)	Light gray
10.1(10C6)	White

Table 6. Overall summary of the frequency of occurrence of different colours of the aerial mycelia of the *Streptomyces* isolates

Colour class	Number of isolates	% of isolates
White	21	20
Gray	61	58.1
Pink	2	1.9
Red	2	1.9
Yellow	2	1.9
Cream	17	16.2
Total	105	100.0

Table 7. Distribution of colours of aerial mycelium of the *Streptomyces* isolates by soil pH level

Soil pH level	Colour class	Number of *Streptomyces* isolates	% of isolates within each pH level
4.5	White	4	80
	Gray	1	20
5.95	White	2	33
	Whitish	1	16.7
	Yellow	1	16.7
	Light yellow	1	16.7
	Cream	1	16.7
6.65	Cream	1	25
	Light yellow	1	25
	Gray white	1	25
	Gray	1	25
7.2	White	9	28.1
	Cream	4	12.5
	Gray	7	21.8
	Grayish	1	3.1
	Whitish	1	3.1
	White gray	9	28
	Light gray	1	3.1
8.2	White	2	4.4
	Gray	3	6.7
	White gray	14	31.1
	Red	1	2.2
	Reddish	1	2.2
	Yellow pink	1	2.2
	Light gray	15	33.3
	Pink	1	2.2
	Cream	7	15.5
10.1	White gray	6	42.8
	White	1	7.1
	Light gray	1	7.1
	Milk white	1	7.1
	Cream	5	35.7

4.4 Characterization of the *Streptomyces* isolates by morphology of their spore chains

Table 8 shows the morphologies of the spore chains of the *Streptomyces* isolates. The distribution of *Streptomyces* isolates according to morphology of aerial mycelium ranged from flexuous to monoverticillate, in different proportions. The flexuous morphological type was dominant (about 60.9%) followed by open spirals, straight, open loops, closed spirals, hooks and monoverticillate. The frequency/extent of occurrence of each morphological type is summarized in Table 8, while the overall summary of occurrence of different morphological types is given in Table 9, and their variation by pH presented in Table 10. The flexuous morphology was also generally dominant at each pH level (with the exception of pH 4.5) followed by open spiral morphology with exception of at pH 6.65 (Table 10). They generally agree with the trend observed in Table 9.

The occurrence of the different morphological types may imply that the different morphological types of *Streptomyces* isolates were adapted to all the pH values used in this study. Different morphologies presently observed signify variability in the *Streptomyces* isolates. The different trends of dominance of different morphological types may imply that each type is adapted more to a particular pH.

Table 8. Morphologies of *Streptomyces* isolates spore chains

pH/ Strain No.	Morphology
4.5(1)	Straight
4.5(2)	Straight
4.5(3)	Straight
4 5(6)	Straight
4.5(41)	Open loops
5.95(16)	Open spirals
5.95(7	Flexuous
5 95(39)	Close spirals
5 95(34)	Flexuous
5.95(10)	Flexuous
5.95(11)	Flexuous
6 65(16)	Straight
6.65(13)	Flexuous
6.65(19)	Hooks
7.2(14)	Flexuous
7.2(15)	Flexuous
7.2(16)	Flexuous
7.2(17)	Flexuous
7.2(18)	Flexuous
7 2(19)	Flexuous
7.2(20)	Flexuous
7.2(21)	Flexuous
7 2(22)	Flexuous
7.2(23)	Flexuous
7.2(24)	Open loop
7.2(25)	Open spirals
7.2(35)	Open spirals
7.2(27)	Flexuous
7 2(12)	Open loops
7.2(29)	Open loops
7.2(4)	Flexuous
7.2(11)	Straight
7.2(32)	Flexuous
7.2(33)	Flexuous
7.2(34)	Flexuous
7 2(35)	Open loops
7.2(36)	Monoverticillate
7.2(37)	Straight
7.2(38)	Open spirals
7.2(39)	Open spirals
7 2(40)	Open spirals
7.2(41)	Straight
7.2(42)	Flexuous
7.2(43)	Flexuous
7.2(44)	Flexuous
7 2(45)	Flexuous
7.2(5)	Hooks
8.2(28)	Open spirals
8.2(58)	Open loops
8 2(48)	Open spirals
8.2(1)	Flexuous
8 2(2)	Flexuous
8.2(3)	Flexuous
8.2(4)	Flexuous
8.2(5)	Flexuous
8.2(6)	Flexuous
8.2(7)	Flexuous
8.2(8)	Flexuous
8.2(9)	Flexuous
8 2(10)	Flexuous
8.2(11)	Flexuous
8.2(12)	Flexuous
8.2(13)	Flexuous

8.2(14)	Flexuous
8.2(15)	Flexuous
8.2(16)	Flexuous
8.2(49)	Flexuous
8.2(50)	Closed spirals
8.2(38)	Open loops
8.2(18)	Flexuous
8.2(19)	Flexuous
8.2(20)	Straight
8.2(21)	Straight
8.2(22)	Open loops
8.2(23)	Closed spirals
8.2(24)	Open spirals
8.2(25)	Flexuous
8.2(26)	Flexuous
8.2(27)	Flexuous
8.2(28)	Flexuous
8.2(29)	Straight
8.2(30)	Flexuous
8.2(31)	Flexuous
8.2(32)	Flexuous
8.2(33)	Flexuous
8.2(34)	Flexuous
8.2(35)	Flexuous
8.2(36)	Flexuous
8.2(37)	Flexuous
8.2(38)	Flexuous
8.2(39)	Flexuous
8.2(40)	Flexuous
10.1(16Q)	Open spirals
10.1(17Q)	Open spirals
10.1(18Q)	Open spirals
10.1(19Q)	Open spirals
10.1(20Q)	Open spirals
10.1(21Q)	Flexuous
10.1(44)	Flexuous
10.1(10C1)	Cream
10.1(10C2)	Flexuous
10.1(10C3)	Flexuous
10.1(10C4)	Flexuous
10.1(10C5)	Straight
10.1(10C6)	Open spirals

Table 9. Overall summary of the frequency of occurrence of the different morphological types of *Streptomyces* isolates

Morphology	Number of isolates	% of isolates
Straight	13	12.4
Open spirals	14	13.3
Flexuous	64	60.9
Closed spirals	3	2.9
Open loops	8	7.6
Monoverticillate	1	0.9
Hooks	2	1.9
Total	105	99.9

Table 10. Distribution of morphologies of *Streptomyces* isolates by pH levels

pH level	Morphology	Number of isolates	% of isolates within each pH level
4.5	Straight	4	80
	Open loops	1	20
5.95	Open spirals	1	100
6.65	Straight	1	25
	Flexuous	2	50
	Hooks	1	25
7.2	Flexuous	19	29.
	Open loops	4	13.8
	Open spirals	5	17.2
	Monoverticillate	1	3.4
8.2	Hooks	1	2.2
	Open spirals	3	6.5
	Open loops	3	6.5
	Closed spirals	2	4.3
	Flexuous	34	74
	Straight	3	6.5
10.1	Open spirals	6	50
	Flexuous	5	42
	Straight	1	8.3

4.5 Evaluation of ranges of tolerance of the *Streptomyces* isolates to pH changes

The ranges of tolerance of the *Streptomyces* isolates to pH changes are given in Table 11. The results show that all isolates from an original soil pH of 4.5 were able to tolerate/grow up to pH 7.0 but failed to grow on media of pH 9.0 and pH 4.0. The isolates from pH 5.9 failed to grow at pH 4.0 and 9.0 but showed some growth at pH 7.0.

The isolates from pH 6.6 showed reasonable growth at pH 5.0 but did not grow at pH 4.0. They also showed minor growth at pH 8.0 and 9.0 but failed to grow at pH 10.1. Isolates from pH 7.2 showed no growth when cultured at pH 4.0 but had minor growth at pH 5.0 and at pH 8.0, but could not grow at pH 9.0.

Isolates from pH 7.85 showed some growth at pH 5.0 but not at pH 4.0. They also showed little growth at pH 9.0. The *Streptomyces* strains isolated from pH 8.2 showed no growth at pH 4.0 and pH 10.0 but some growth was observed at pH 5.0 and 9.0. The *Streptomyces* isolates from the very extreme condition of alkalinity (pH 10.1) showed poor growth at that particular pH value. However, they did well at pH 7.0 with some growth at pH 8.0, 9.0 and pH 5.0. However, they could not grow at all when cultured at pH 4.0.

Table 11. The range of pH tolerance of *Streptomyces* isolates

Original soil pH	Tested pH	Relative growth of *Streptomyces*	Number of isolates grew in each tested pH level
4.5	4.0	+	1 (out of 10)
	4.5	++	1
	5.0	+++	10
	7.0	+++	10
	9.0	0	10
5.95	4.0	0	10 (out of 10)
	5.95	+	10
	7.0	+	10
	9.0	0	10
6.65	4.0	0	15 (out of 15)
	5.0	++	15
	6.65	+++	15
	8.0	++	15
	9.0	0	15
	10.0	0	15
7.2	4.0	0	15 (out of 15)
	5.0	+	3
	7.2	+++	15
	8.0	+	15
	9.0	0	15
7.85	4.0	0	20 (out of 20)
	5.0	+	20
	7.0	+++	20
	7.85	+++	20
	9.0	+	1
8.2	4.0	0	15 (out of 15)
	6.0	+++	15
	8.2	++	15
	10.0	0	15
10.1	4.0	0	20 (out of 20)
	5.0	+	20
	7.0	+++	20
	8.0	++	20
	9.0	+	20
	10.1	+	20

Growth scores:

+ + + =very good,

+ + = Good,

+ = Fair,

0 = No growth at all.

The failure of isolates to grow at very low or very high pH levels may be due to different mechanisms or metabolic processes. The very extreme pH (low or high) might be not favourable to different enzyme systems or metabolic activities that are required for the survival of *Streptomyces*. On the other hand, the reasons for the ability of the few isolates to grow at pH 4.5, 8.2 and 9.0 (Table 11) may be due to some special mechanisms which enabled adaptation of those isolates at those particular pH levels. For example, Krulwich and Guffanti, (1989) found that the properties affected adaptation of organisms to higher pH values were compositions of membrane lipids and the membrane lipid/protein ratio, very high levels of respiratory chain metabolic components in membranes, composition of proteins, a Na^+ cycle that facilitates solute uptake and pH homeostasis. Millard and Taylor (1927 as cited by Taber, 1960) reported that some isolates produced glutamate dehydrogenase, an enzyme which is responsible for ammonia assimilation and glutamate catabolism, and observed that these reactions were optimal at pH 8.2 and 9.2, respectively.

The ability of all *Streptomyces* isolates tested to grow at pH 6.6 or 7.2 implies that these pH ranges were conducive for the functioning of different metabolic/enzymes systems to enable proliferation of the *Streptomyces*. Many *Streptomyces* were reported to produce chitinase enzymes (at pH 7.0) and that chitin addition provided them with a readily available source of nutrients (Jeauniaux, 1955; Lingappa and Lockwood, 1961; 1962 cited by Davies and Williams, 1970).

The low pH in the present soils might have prevented *Streptomyces* growth by several mechanisms. At low pH some nutrients may be accessible to *Streptomyces* cells while others may not be. Thus, after exhaustion of nutrient reserves growth ceases. Taber (1960) reported that assimilation of glucose prevented by low pH due to inability of cells to respire exogenous glucose, and this eventually reduced metabolic activities. Williams and Mayfield (1970) also found that *Streptomyces* populations decreased as the soil became more acidic, but it was not until pH dropped below 4.2 that populations reached zero.

Generally, observations from the present study show that while *Streptomyces* isolated from conditions of extreme acidity (pH 4.5) were able to grow even on the alkalinity side, the *Streptomyces* isolates from extreme alkalinity could not grow under very acidic conditions. The results also show that all isolates (both from extreme acidity and extreme alkalinity) were found to proliferate well when cultured at pH levels between 6.6-7.8. This agrees with Alexander (1983) and Crawford *et al.* (1983) who observed that *Streptomyces* preferred neutral to slightly alkaline condition as compared to acidic conditions.

4.6 Evaluation of the *Streptomyces* isolates for antimicrobial activity against bacterial plant pathogens *in vitro*

4.6.1 General

The ability of selected *Streptomyces* isolates to inhibit the growth of some plant pathogenic bacteria is shown in Table 12. The pH values used for antibiosis testing were selected based on results of ranges of pH tolerance (see section 4.5 and Table 11). Because all isolates, including those from extreme acidity and alkalinity, were found to grow well at pH 6.6 and 7.2, the pH values 4.5, 5.9, 6.6 and 7.2 were selected for antibiosis testing. Where more than one isolate was available from any particular original soil pH, all were tested in the whole range of tested pH. Where only one isolate was available, it was also tested as such.

Table 12. The extent of antibiosis activity (mm of inhibition) of some *Streptomyces* isolates towards some plant pathogens as influenced by pH

Original soil pH	Tested medium pH(isolate)	Pathogens					
		Xanthomonas phaseoli	*X. oryzae* pv *oryzae*	*Clavibacter michiganensis* sub sp *michiganensis*	*X. vascatoria*	*X. phaseoli-coli* var *fuscoris*	*Acidovorax avenae*
4.5	4.5(1)	13	13	13	6	0	10
	5.95(1)	6	0	16	0	0	0
	6.6(1)	0	6	32	0	0	0
	4.5(41)	12	13	35	12	0	33
	5.95(41)	6	12	40	9	0	13
	7.2(41)	11	0	32	32	0	0
	4.5(6)	12	13	13	12	0	33
	5.95(6)	12	6	32	8	0	25
	7.2(6)	6	23	45	22	0	0
5.95	5.95(10)	12	0	32	0	0	12
	7.2(10)	10	0	33	0	0	0
	5.95(16)	9	8	32	7	0	22
	6.6(16)	6	11	35	9	0	0
	5.95(34)	13	0	37	6	0	13
	7.2(34)	11	11	34	0	0	0
	5.95(39)	0	12	33	6	0	14
	7.2(39)	13	15	34	6	0	0
	6.6(16)	6	11	35	9	0	0
	6.6(19)	0	10	33	11	0	0
7.85	6.6(2)	12	7	23	0	0	0
8.2	6.6(13)	0	0	0	0	0	0
6.6	6.6(7)	8	16	30	0	0	0
8.2	7.2(17)	0	11	42	25	0	0
8.2	7.2(18)	12	0	0	0	0	0
7.2	7.2(5)	16	0	42	12	0	15
8.2	7.2(44)	13	0	33	13	0	12
10.0	7.2(22)	13	13	23	13	0	0
7.85	7.2(14)	21	6	45	23	0	0
7.85	7.2(12)	13	0	33	33	0	11
10.0	7.2(20)	0	0	33	0	0	0
8.2	7.2(35)	0	0	0	0	0	0
7.85	7.2(36)	0	11	15	0	0	0
8.2	7.2(11)	0	0	0	0	0	0
7.2	7.2(25)	13	5	45	33	0	0
10.0	7.2(26)	13	6	43	33	0	0
7.2	7.2(4)	0	O	0	0	0	0
7.2	7.2(23)	13	23	45	11	0	0
8.2	7.2(7)	0	0	0	0	0	0

0= no antibiosis, 10= weak antibiosis, 11-25= moderate antibiosis, >25= strong antibiosis

Some isolates showed strong inhibition of the tested plant pathogens (>25mm of inhibition zone), others moderate (11 to 25mm) while others exhibited weak inhibition (<10mm). A few isolates did not show any antibiosis activity against any of plant pathogens tested. For some isolates (Table 12) the antibiosis increased with increase in medium pH, for example isolate number 1 and 6 against *C. michiganensis* sub sp.*michiganensis.* For other isolates, for example no.1 against *X. phaseoli* and no.41 against *A. avenae*, antibiosis decreased with increase in medium pH. In other isolates there was generally no major changes when pH changed, for example isolate no. 10 against *C. michiganensis* sub sp.*michiganensis.*

In Table 12, the plant pathogen *Acidovorax avenae* was inhibited by the least number of *Streptomyces* isolates especially those isolated from acidic soils (pH 4.5 and 5.9) and was generally not inhibited when observed at media pH 6.6 and 7.2. However, three isolates from extreme alkalinity condition, namely 7.2(5), 7.2(44) and 7.2(12) showed moderate antibiosis against *Acidovorax avenae.*

These results of antibiosis for acidophillic *Streptomyces* were obtained when the isolates were tested at their original pH value(4.5 and 5.9). However, when tested at high pH, the isolates showed generally weak antibiosis against most of the test pathogens, with exception of against *C. michiganensis* sub sp *michiganensis.*

Isolates from extreme alkalinity were generally found to be moderate to strong antibiotic producers. Generally, wherever isolates grew well within the range of pH tol-

erance (Table 11), they were also found to do well in antibiosis, though at various extents. The changes in the extent of antibiosis at different pH levels may imply that antibiotic production changes as pH changes. This may be attributed by inherent sensitivity of the tested plant pathogens towards the antibiotics produced by different isolate(s). Each isolate may produce antibiotic that is more sensitive to either of the plant pathogen than another. It is also possible that those isolates produced more than one antibiotics which might have different sensitivity to the pathogens tested. This agree with Brock *et al.* (1994) who reported on the possibilities of some *Streptomyces* species to produce more than one antibiotic and that, often several kinds produced may not necessarily be chemically related. For example, isolate 5.95(34) did not show any antibiosis against *X. oryzae* pv oryzae but showed moderate antibiosis against *X. phaseoli* and *A. avenae* and showed strong antibiosis against *C. michiganensis* sub sp *michiganensis* and weak antibiosis against *X. vascatoria*. Isolate 7.2(10) showed strong antibiosis against *C. michiganensis* sub sp *michiganensis*, weak antibiosis against *X. phaseoli* and did not show any antibiosis against the rest of the *Streptomyces* isolates.

The increase in antibiosis with the increasing pH in some *Streptomyces* isolates may imply different things. Firstly, those antibiotics produced may have been more active/potent in alkaline conditions than acidic conditions. Secondly, it is also possible that antibiotics produced by those isolates were not the same, meaning that different antibiotics were synthesized at different pH. Therefore, increasing the pH might have created favourable environments for particular isolates to produce particular antibiot-

ics that could not be synthesized in acidic conditions. El-Talabily *et al.* (1996) reported that as pH was increased by liming to 6.9 a variety of antibiotics were produced.

The decrease in antibiotics with increasing pH may mean that antibiotics produced against those plant pathogens were active at low pH. Therefore, increasing the pH might have created an environment in which antibiotic activity decreased.

The decrease in antibiosis, followed by its increase with the pH rise (e.g. isolate 6 against *X. vascatoria and X .oryzae* pv oryzae), may imply that synthesis and/or activity of some of these antibiotics at first decreased with increasing pH. The resumed high antibiosis at higher pH may imply that different antibiotic(s) was synthesized at the higher pH.

Lack of any changes in antibiosis for some isolates when the pH changed may imply that those isolates might have produced an antibiotic(s) that may be well adapted to all pH ranges tested. Therefore, increasing or decreasing the pH could not resulted in any significant change on antibiosis characteristics.

Sensitivity of *Acidovorax avenae* to fewer *Streptomyces* isolates may be explained using different scenarios. Firstly, it is possible that this plant pathogen was insensitive to all the *Streptomyces* isolates. It is also possible that the tested *Streptomyces* isolates produced antibiotics which were not effective against this plant pathogen.

4.6.2 Overall summary of antibiosis activity of the *Streptomyces* isolates against plant pathogens

The overall summary of antibiosis activity is given in Table 13. Only six isolates did not show any antibiosis against any of the tested plant pathogens. Twenty five isolates showed weak antibiosis, fifty eight were moderate while thirty one isolates showed strong antibiosis.

From Table 13, the failure of six isolates (about 16.2% of all isolates) to show any antibiosis towards the test plant pathogens may imply that either those isolates were not antibiotic producers. It is also probable that they might have produced antibiotics which were not effective against the tested organisms but may be effective against other pathogens not tested at present.

Table 13 Summary of antibiosis activity of the *Streptomyces* isolates

Extent of inhibition, mm	Number of *Streptomyces* isolates showing antibiosis	% of isolates
None	6	5
1-10mm (weak)	25	22.9
11-25mm (moderate)	58	53.2
>25mm (strong)	31	28.4

4.6.3 Summary of antibiosis activity by of the *Streptomyces* isolates by pH levels

The summary of antibiosis activity by pH levels is given in Table 14. The results indicate that pH 7.2 resulted in antibiosis by most isolates.

The antibiosis behaviour of a greater proportions of the isolates at pH 7.2 may imply that pH 7.2 might offer the conditions most favourable for the production and stability of antibiotics produced. This agrees with Hays *et al.* (1948) who reported that pH 6.8 was optimum for the growth of *Penicillium* while pH 7.3 was best for penicilin formation. However, it should be kept in mind that possibilities exist for maximal production of an antibiotic at other pH levels. Evidence pointing this was observed in section 4.6.1 whereby some *Streptomyces* isolates (e.g. no. 6 and 4) seemed to show high production even on the acidic side of the pH scale.

Table 14. Summary of antibiosis by pH levels

pH tested	Number of isolates showing antibiosis	% of isolates
4.5	15	13.8
5.95	28	25.7
6.6	13	11.9
7.2	53	48.6
Total	109	100.0

4.6.4 Categorization of the *Streptomyces* isolates' antibiosis by extent of antibiosis

The separation of the *Streptomyces* by extents of antibiosis is given in Table 15. Most isolates showed moderate antibiosis against all test plant pathogens. Fewer isolates had strong antibiosis, or weak antibiosis, but against fewer of the tested plant pathogens. *Clavibacter michiganensis* sub sp.*michiganensis* showed strong and moderate inhibition.

The groupings of *Streptomyces* isolates into strong, moderate and weak antibiosis may imply that the sensitivity of the plant pathogens towards the antibiotics produced differed. While some pathogens may be both strongly and moderately inhibited, others may be moderately to weakly inhibited. This implies that some of the *Streptomyces* isolates might have produced antibiotics with a wide range of activity. The antibacterial activity of some antibiotics may have been higher and widely distributed while it may have been lower in other antibiotics.

Apparent sensitivity of *C. michiganensis* sub sp *michiganensis* to almost all of the isolates may indicate several things. First, most or all of those *Streptomyces* isolates may have produced similar antimicrobial compound(s) to which *C. michiganensis* sub sp *michiganensis* was sensitive. This agrees with Brork *et al.* (1994) who reported on the ability of different species of *Streptomyces* to produce similar antibiotics.

Table 15. The categorization of different *Streptomyces* isolates by extent of antibiosis

Antibiosis status	Plant pathogens inhibited	Number of isolates showing antibiosis	% of isolates in each antibiosis category
Strong antibiosis	*Clavibacter michiganensis* sub sp *michiganensis*	25	80.6
	Acidovorax avenae	2	6.4
	X. vascatoria	4	12.9
	Total	31	99.9
Moderate antibiosis	*X. phaseoli*	19	32.7
	X. oryzae pv oryzae	14	24.1
	C. michiganensis sub sp *michiganensis*	6	10.3
	A. avenae	9	15.5
	X. vascatoria	10	17.2
	Total	58	99.8
Weak antibiosis	*X. phaseoli*	8	32.0
	X. oryzae pv oryzae	8	32.0
	X. vascatoria	8	32.0
	A. avenae	1	4.0
	Total	25	100.0

Similarly, Lechevalier *et al.* (1970) also found that similar actinomycetes tended to produce similar families of antibiotics. Secondly, it may also indicate that those isolates produced different antibiotics and the plant pathogens themselves were sensitive to almost all the antibiotics produced.

Generally, about 83.8% of all isolates tested were observed to have the ability to inhibit the growth of one or the other plant pathogens tested though at different proportions. This is in agreement with the conclusion by Alexander (1977) that over 75% of all soil *Streptomyces* were capable of producing antibiotics. Similarly, Porter *et al.* (1971) observed that nearly all *Streptomyces* are able to produce antibiotics. The results also indicate that the *Streptomyces* isolates with antimicrobial potential can be obtained from extreme conditions of acidity and alkalinity. This calls for further attention on these extreme environments in future.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY AND CONCLUSIONS

The study reported herein revealed low *Streptomyces* populations at pH 4.5 and 10.1 ($\log_{10}$ values of 3.2 and 3.5, respectively). The highest *Streptomyces* population with a mean of 5.4 was encountered at pH 7.2. The study revealed *Streptomyces* of different colours of aerial mycelium which ranged from gray to white to cream, red and pink types and their intergrades, in different proportions. The gray series dominated all other colours.

Different morphological groups were also encountered whereby the flexuous morphological type was dominant (about 60%) followed by open spirals, straight, open loops, closed spirals, hooks and monoverticillate. The ranges of tolerance of different *Streptomyces* isolates showed that all isolates from an original soil of pH 4.5 were able to tolerate/grow up to pH 7.0 but failed to grow at pH 9.0 and 4.0. The isolates from very extreme conditions of alkalinity (pH 10.1) showed poor growth at that particular pH value but did well at pH 7.0 with some growth at pH 8.0, 9.0 and 5.0. However, they did not grow at all when cultured at pH 4.0. Generally, all isolates were able to grow between pH 6.6 and 7.8.

About 84% of the isolates produced antibiosis against the tested plant pathogens. Six isolates (about 16.2% of all isolates) did not produce antibiosis against any of the tested plant pathogens. On the other hand, the plant pathogen *X. phaseolicoli* var fuscoris was not inhibited by any of the isolates tested.

To conclude, this study revealed *Streptomyces* of different morphological groups and colours of mature colonies which were potential antibiotics producers. It should be kept in mind that although some isolates did not produce antibiotics here, they may do so if tested with other pathogens which were not included in this study. Therefore, the status of antibiosis of different isolates reported herein should be taken as a challenge for further studies.

5.2 RECOMMENDATIONS

Based on the findings in the current study, it is recommended that:-

(1) Further studies be undertaken on extreme conditions of acidity and alkalinity to reveal more species which might be of great antibiotic potential,

(2) Studies to be carried out to extract antibiotics from *Streptomyces* and test their effectiveness under field conditions,

(3) Classifications of *Streptomyces* to species level should be undertaken for clearly understanding of these microorganisms from our soil environments.

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