CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF *ACTINOMYCETES* ISOLATED FROM SELECTED SITES IN LAKE VICTORIA SHORES

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A THESIS SUBMITTED TO THE BOARD OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE IN MASTER OF SCIENCE IN MICROBIOLOGY OF JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DECLARATION AND APPROVAL

DECLARATION

This thesis is my original work and has not been presented for award of a degree or diploma in any University or institution.

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RECOMMENDATIONS

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DEDICATION

This thesis is dedicated to my beloved husband Mr. Richard Okoth, my daughter Eunice and my sons Christian Shama, Emmanuel Otieno and Dennis Mich whose constant encouragements and support propelled me to pursue further studies. I also dedicate it to Mum Lilian and Elma for their spiritual encouragements. All of them inspired me towards my academic work through prayers and encouragement.

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ABSTRACT

The global threat of antibiotic resistance necessitates the discovery of novel antibiotics to combat drug-resistant bacterial pathogens. *Actinomycetes*, known for their bioactive compound production, offer a promising solution. This study evaluated the growth of *Actinomycetes* from Winam Gulf of Lake Victoria shores on various media, screened them for antibacterial activities, and characterized bioactive species using morphological, Biochemical and molecular markers. Eight sampling sites (Mbita, Asembo Bay, Kunya Beach, Kendu Bay, Usenge, Luanda Kotieno, Homa Bay, and Kisumu) along Winam Gulf were randomly selected. Systematic random sampling identified three sampling points (1 m in-shore) from a random starting point, with 10 m interval between points. Soil sediments were collected in triplicate from each site, giving a total of 24 samples, and transported to JOOUST Botany Laboratory. In-vitro cultural growth was assessed on Yeast Malt Extract Agar (YMEA), Soya Casein Agar, Peptone B Agar, and Yeast Extract Agar using serial dilution method. Colony size was measured on day 10. Primary screening of antibacterial activity of 16 pure isolates was done on Mueller Hinton Agar against *Staphylococcus spp.*, *Streptococcus spp.*, *Shigella spp.*, *Xanthomonas spp.*, and *E. coli* obtained from Kenya Medical Research Institute, Kisumu. *Actinomycetes* isolates were characterized morphologically, biochemically, and molecularly. Data on growth and antibacterial activity were analyzed using SAS version 21 with one-way ANOVA at 95% confidence level and means separated by Least Significant Difference ($p \leq 0.05$). Sequences were assembled using BioEdit software aligner and transferred to MEGA Version 6.0 and aligned using CLUSTAL W. Individual consensus sequences of the 16S rRNA gene regions were used to evaluate closely related sequences at the NCBI GenBank using BLAST. YMEA showed the highest growth with isolate A3 from Asembo Bay at 14.67 mm. Out of 16 pure isolates, 9 exhibited bioactivities against test pathogens. Isolate MT3 (*Streptomyces microflavus*) from Mbita demonstrated the highest antibacterial activity. Molecular characterization revealed limited genetic diversity among Winam Gulf *Actinomycetes*, predominantly within the *Streptomyces* genus, including *Streptomyces microflavus* (MT3 and KB3), *Streptomyces celluloflavus* (U3), *Streptomyces werraensis* (K3), and *Streptomyces cellulosae* (KSM3). Isolates MT3 and K3 are recommended for further research due to their potential in producing potent drugs against pathogenic bacteria. This study's findings contribute to academic knowledge and practical applications in combating antibiotic resistance, addressing a critical global health challenge.

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LIST OF ACRONYMS AND ABBREVIATIONS

AMR	Antimicrobial Resistance
ANOVA	Analysis of Variance
BLAST	Basic local Alignment Search Tool
CDDEP	Center for Disease Dynamics Economic Policy
DNA	Deoxyribonucleic Acid
ECDPC	European Centre for Disease Prevention and Control
EMA	European Medicine Agency
G + C	Guanine + Cytosine
KEMRI	Kenya Medical Research Institute
LSD	Least Significance Difference
MHA	Mueller Hinton agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
PVC	Polyvinyl chloride
rDNA	Recombination DNA molecules
rRNA	Ribosomal RNA
16 S rRNA	Svedberg unit ribosomal Ribonucleic acid
WHO	World health organization
YMEA	Yeast-Malt Extract Agar

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Actinomycetes are a group of filamentous Gram + bacteria which produce useful pharmaceutical by-products including, antibiotics, antifungals, and antitumor agents (Al-Dhabi *et al*., 2016; Barka *et al*., 2016; Muthu *et al*., 2013). *Actinomycetes* thrive in diverse environments such as soil and aquatic habitats, where they play a crucial role in natural product synthesis (Ezeobiora *et al*., 2022). They are prolific producers of secondary metabolites critical for pharmaceutical and industrial applications, significantly contributing to global health by providing essential antibiotics (Ezeobiora *et al*., 2022). These microorganisms are responsible for synthesizing approximately two-thirds of all known antibiotics, essential in combating bacterial infections and addressing the escalating challenge of antibiotic resistance (Barka *et al*., 2016). The challenge of antibiotic resistance is an emerging global health threat, which has slowed the attainment of the United Nations Sustainable Development Goals of reduced morbidity, mortality, and healthcare costs associated with resistant infections (O'Neill, 2016). Increased use of antibiotics by humans and animals has led to multi-drug resistance of infectious agents (Center for Disease Dynamics Economic Policy, 2015). The number of multi-resistant bacterial strains has increased at an alarming rate, and finding therapy that ensure effective treatment remains a challenge for physicians (Tarai *et al*., 2013). The most common causes of resistance include poor usage of antibiotics, including non-compliance to dosage prescriptions, selection pressure due to overuse of antibiotics, and the transfer of resistant bacteria between patients or from a health worker to a patient (Aly and Balkhy, 2012; Huttner *et al*., 2013). Animals raised with antibiotics host important populations of antibiotic-resistant bacteria, which are transmitted to humans through their products or direct contact with the animals (Andersson and Hughes, 2017). Globally, up to 700,000 people die yearly from drug-resistant infections, and this may reach 10 million by 2050 if the present trend persists (O'Neill, 2016). The heavy burden of transmissible diseases, especially HIV and AIDS, implies widespread antimicrobial use and subsequent resistance, as shown in the World Health Organization's (WHO) global report on antimicrobial resistance among clinically significant bacterial isolates in sub-Saharan Africa (Leopold *et al*., 2014). The discovery of new bioactive compounds, especially those with new modes of action, is not only needed for current medicine but is also essential to evade future pandemics (Jakubiec-Krzesniak *et al*., 2018). A high percentage of hospital-acquired infections are caused by highly resistant bacteria such as

methicillin-resistant *Staphylococcus aureus* (MRSA) (WHO, 2015). *Staphylococcus aureus*, *Escherichia coli, Vibrio cholerae, Enterobacter* species, and *Salmonella enterica* are some of the multi-drug resistant developing bacteria (Andersen *et al*., 2015). Resistance to antibiotics has rapidly increased, prompting researchers to shift focus to discovering elite and potent bioactive compounds originating from *Actinomycetes* found in natural sources, such as aquatic environment of Winam Gulf, Lake Victoria, that could be used to treat infections caused by microorganisms (Sebak *et al*., 2021). Most antibiotics are either partial synthetic derivatives or natural products from fungi or *Actinobacteria* (Valli *et al*., 2012). *Actinomycetes* are sources of more than 45% of the world's microbial bioactive products, making them the most important prokaryotes economically (Subathra *et al*., 2022). Metabolites from *Actinomycetes* have valuable capabilities, including the production of immune modulators, antibiotics, cosmetics, enzyme inhibitors, antitumor agents, and enzymes (Subathra *et al*., 2022). Indeed, several antibiotics have been discovered from *Actinomycetes*, including rifamycin, erythromycin, pacidamycin, caprazamycin, capuramycin, and thiolactomycin (de Souza, 2009). The most studied genus has been *Streptomyces*, which is capable of producing secondary metabolites that are naturally bioactive. Most of these metabolites are antimicrobials, enzyme inhibitors, enzymes, anti-cancer agents, antioxidants, and many others (Barka *et al*., 2016; Sharma and Thakur, 2020). Recently, researchers have noted an increase in the number of *Streptomyces* isolated and identified them as superior compared to other *Actinomycetes* strains due to their ability to produce a wide range of bioactive secondary metabolites (Nandhini *et al*., 2015). *Streptomyces* produce about two-thirds of all naturally derived antibiotics currently used in veterinary practice, medicine, and agriculture (Barka *et al*., 2016).

Taxonomically, *Actinomycetes* are filamentous, free-living, or saprophytic, Gram-positive bacteria found in organically rich soils, fresh or marine water, and as endophytes (Barka *et al*., 2016). Screening *Actinomycetes* for bioactive compound production requires exploring new natural sources, assessing their in vitro culture growth requirements, and evaluating species diversity (Bull and Stach, 2007). Screening tests are conducted to identify antimicrobial agents that are abundantly expressed and have easily visualized zones of inhibition (Byrne, 2020). However, a drastic decrease in new antibiotics discovery has been reported recently due to extensive previous screening from soil sources and the uneconomic re-discovery of already known compounds (Zainal *et al*., 2016). Therefore, exploring previously untapped sites with the potential for new antibiotic-producing *Actinomycetes* is necessary. Such unexplored sources include organically rich sediments of large freshwater bodies, such as Lake Victoria. Despite facing environmental

challenges from anthropogenic activities leading to pollution and eutrophication (Simiyu *et al*., 2018; Ogello *et al*., 2013), Lake Victoria's sediments offer an intriguing and under explored niche for discovering novel *Actinomycetes* species with potential bioactive properties.

Winam Gulf, a part of Lake Victoria, presents a unique environment that could harbor distinct *Actinomycetes* strains due to its specific ecological conditions. This gulf is characterized by varying degrees of pollution and organic matter deposition, creating a fertile ground for *Actinomycetes* with the potential to produce novel bioactive compounds (Mwamburi,2016). The sediments of Winam Gulf are particularly rich in organic material, making it an ideal site for isolating *Actinomycetes* (Simiyu *et al*., 2018). Therefore, this study focuses on *Actinomycetes* from Winam Gulf as a representative site within Lake Victoria to evaluate their bioactive potential.

In natural habitats, *Actinomycetes* are free-living, anaerobic, and require organically rich environments (Takahashi and Nakashima, 2018; Bizuye *et al*., 2013), such as the sediments of Lake Victoria. Despite their potential, *Actinomycetes* from Lake Victoria sediments remain understudied for their bioactive potential.

To utilize *Actinomycetes* from natural sources for antibacterial compounds, it is crucial to establish the *in vitro* capacity to grow on synthetic media. One of the most important factors determining the growth of *Actinomycetes* is the availability of nutrients. Majority of *Actinomycetes* can use a wide range of compounds such as glucose, amino acids, proteins, and starch as their energy source, unlike other bacterial groups that only utilize simple carbon and nitrogen sources (Bhatti *et al*., 2017). Production of bioactive metabolites by *Actinomycetes* is greatly dependent upon the most suitable in vitro cultural conditions for mycelial growth and proliferation (Arul *et al*., 2011; Wang *et al*., 2010). Establishing a suitable culture medium is an important prerequisite for studying and commercializing *Actinomycetes* bioactive products (Ganeshamurthy *et al*., 2021). Previous studies suggested that different combinations of media could be used for isolating *Actinomycetes* (El-Karkouri *et al*., 2019; Schneegurt, 2012). Bawazir *et al*., (2018) successfully cultivated *Actinomycetes* on starch casein agar, yeast-malt extract agar, and glycerol yeast extract agar. Little work has been done to establish in vitro growth requirements for *Actinomycetes* isolated from sediments Winam Gulf of Lake Victoria shores.

Analysis of potential bioactive metabolites from *Actinomycetes* involves primary and secondary screening (Charousová *et al*., 2017). Primary screening requires the isolation and identification of pure *Actinomycetes* species using the cross-streak method on media plates (Sibanda *et al*., 2010).

The isolates are then subjected to secondary screening for antibacterial activities against selected drug-resistant bacterial strains using perpendicular streak and agar overlay methods (Mohanraj *et al*., 2011), especially if the isolates are required for further studies (Mohan *et al*., 2014). In Kenya, bioprospecting for broad-spectrum antibiotic-producing *Actinomycetes* has been conducted from virgin soils in Kericho County (Rotich *et al*., 2017), Menengai Craters (Waithaka *et al*., 2017), waste dump sites in Thika town (Bizuye *et al*., 2017), and water sediments in Lake Magadi (Ronoh *et al*., 2013). So far, there are no records of studies that have been conducted to assess the bioactive potential of *Actinomycetes* from organically rich sediments of Lake Victoria's shores.

Identification of successfully cultivated *Actinomycetes* requires characterization studies using morphological, biochemical and molecular techniques (Kumari *et al*., 2013). Macroscopic morphology involves mycelial colony growth features such as color on a substrate and production of diffusible pigments (Zothanpuia *et al*., 2018). Microscopic identification is used to establish mycelia fragmentation on the substrate, presence of sclerotia, chain morphology of spores, aerial mycelium and surface ornamentation of spores (Hazarika and Thakur, 2020). Bergey's Manual of Determinative Biochemical Characterization of *Actinomycetes* has listed several techniques of biochemical identification including sodium chloride resistance, catalase test, nitrate reduction, oxidase test, casein hydrolysis, urea hydrolysis, citrate utilization, gram reaction, temperature tolerance, starch hydrolysis and acid production from sugar (Holt *et al*., 2000).

Previous studies on morphological and biochemical analysis of *Actinomycetes* have revealed confounding features, making the techniques taxonomically insufficient (Simeis and Serra, 2021). The most robust technique for identification of *Actinomycetes* is molecular analysis of partial segments of its genomic DNA, such as the small ribosomal sub-unit (16S rRNA) gene (Adegboye and Babalola, 2012). Molecular sequences of the 16S rRNA gene can then be used to undertake phylogenetic analysis to determine the relationships between the isolates (Tatar, 2021). 16S rRNA gene is the most appropriate for studying phylogenetic relationships since it consists of 1542 base pairs which are highly conserved amongst the *Actinomycetes* and other groups of bacteria (Rotich, 2018). This study combined morphological, biochemical and molecular techniques to screen *Actinomycetes* from sediments of Winam Gulf in Lake Victoria shores for antibacterial activity.

1.2 Problem Statement

The rapid rise in antibiotic resistance globally has led to an urgent demand for novel antibiotic sources (Jakubiec-Krzesniak *et al*., 2018). Each year, drug-resistant infections claim the lives of up to 700,000 people worldwide, a number that could escalate to 10 million by 2050 if current trends continue (O'Neill, 2016). Exploring new environments can potentially yield *Actinomycetes* with the capacity to produce rare and unique antibiotic compounds with greater efficacy (Bull and Starch, 2007). Aquatic ecosystems, such as those found in Winam Gulf of Lake Victoria, offer unique environmental conditions compared to terrestrial environments, providing a promising avenue for discovering new *Actinomycetes* (Qadri *et al*., 2020).

However, there has been few studies evaluating *Actinomycetes* from the sediments along the shores of Winam Gulf, Lake Victoria for their capacity to produce antibiotic metabolites. These organically rich sediments remain largely unexplored and may harbor novel strains with unique antibiotic gene clusters and significant clinical potential. To effectively utilize *Actinomycetes* from natural sources like the shores of Lake Victoria for antibacterial purposes, it is critical to establish the *in-vitro* growth on synthetic media and to thoroughly screen and characterize their antibacterial properties (Hug *et al*., 2018). Currently, there is limited data on the diversity of *Actinomycetes* from Lake Victoria sediments and the antimicrobial properties of their metabolites. Addressing this gap, by isolating and evaluating *Actinomycetes* from these aquatic habitats, could lead to the discovery of antibiotics with significant economic and clinical importance (Jagannathan, 2021). Therefore, it is essential to focus on the characterization and screening of *Actinomycetes* from Winam Gulf of Lake Victoria sediments for their antibacterial activity.

1.3 Justification

The research gap in characterizing bioactive *Actinomycetes* is underscored by the urgent need for novel bioactive compounds in the face of increasing multidrug-resistant bacteria. As highlighted by Song *et al*., (2021), the rise in drug-resistant pathogens, including those resistant to commonly used antibiotics derived from *Actinomycetes,* necessitates the exploration of new sources and compounds. This urgency is further emphasized by Mast and Stegmann (2019), who noted the resistance of pathogens to various antibiotic classes previously isolated from *Actinomycetes.*

Moreover, Kumari *et al*., (2013) advocate for improved cultivation strategies, advanced screening techniques, and deeper insights into *Actinomycetes*' genetics and biochemistry to facilitate the

discovery of new antibiotic drugs. This aligns with the proposed objective to characterize bioactive *Actinomycetes* comprehensively.

The significance of the study lies in its potential to contribute to the continuous production of antibacterial drugs by identifying locally sourced *Actinomycetes* with potent antibacterial activity. By isolating and screening *Actinomycetes* from previously unexplored sources like the Winam Gulf sediments of Lake Victoria, researchers can uncover novel strains capable of producing bioactive compounds effective against resistant pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholera*, *Enterobacter species*, and *Salmonella enterica*. Additionally, comparison of locally isolated strains with existing databases based on morphological and molecular markers offers the prospect of identifying superior strains for further development by the pharmaceutical industry. Thus, elucidating the growth potential, bioactivity, and diversity of *Actinomycetes* from Lake Victoria sediments is not only scientifically significant but also holds promise for addressing the pressing global health challenge of antibiotic resistance.

1.4 Objectives

1.4.1 Broad Objective

To evaluate *in-vitro* growth and antibacterial activity of *Actinomycetes* from sediments of Lake Victoria shores and to characterize selected bioactive isolates

1.4.2 Specific Objectives

1. To evaluate *in-vitro* growth of *Actinomycetes* from sediments of Lake Victoria shores on different media
2. To screen *Actinomycetes* obtained from sediments of Lake Victoria shores for antibacterial activity against selected pathogenic bacteria
3. To characterize *Actinomycetes* with antibacterial activity using morphological, biochemical and molecular markers

1.5 Hypothesis

1. Varying nutritional media sources have no significant effect on *in-vitro* growth of *Actinomycetes* from shores of Lake Victoria
2. *Actinomycetes* isolates from the shores of Lake Victoria have no significantly different antibacterial activity against selected test microorganisms
3. *Actinomycetes* species isolated from sediments of Lake Victoria shores are not diverse

1.6 Significance of the study

The study on *Actinomycetes* isolated from Winam Gulf of Lake Victoria sediments significantly supplements existing literature in several key areas. *Actinomycetes* are renowned for their ability to produce bioactive compounds, including antibiotics crucial for combating multidrug-resistant pathogens (Simeis and Serra, 2021). Previous research predominantly focuses on *Actinomycetes* sourced from terrestrial environments, with limited exploration of aquatic habitats such as Lake Victoria (Hug *et al*., 2018). By targeting this unique ecosystem, this study expands the scope of known *Actinomycetes* diversity and bioactivity, contributing novel findings to the field of natural product discovery (Jakubiec-Krzesniak *et al*., 2018).

The findings of this study hold promise for significant advancements in public health and pharmaceutical industries. Antibiotic resistance remains a pressing global health issue, with conventional treatments increasingly ineffective against multidrug resistant bacterial infections (O'Neill, 2016). The discovery of novel bioactive compounds from *Actinomycetes* isolated from Lake Victoria sediments offers potential new avenues for developing effective antimicrobial agents (Bizuye *et al*., 2017). These compounds may provide alternative treatments for infections that are currently difficult to manage, thereby improving patient outcomes and reducing healthcare costs associated with prolonged treatments and hospital stays (Center for Disease Dynamics Economic Policy, 2015).

Furthermore, the optimization of culture conditions and characterization techniques detailed in this study enhances the feasibility of large-scale production and commercialization of these bioactive metabolites (Rotich *et al.*, 2017; Simeis and Serra, 2021). This could lead to the development of new antibiotics, antifungals, and other therapeutic agents with diverse applications in medicine, agriculture, and industry (Barka *et al*., 2016). This study not only enriches scientific understanding of *Actinomycetes* biodiversity in Lake Victoria sediments but also provides actionable insights into their potential as sources of novel bioactive compounds. By elucidating the phylogenetic relationships and biochemical capabilities of these microorganisms, the study lays the groundwork for future research and biotechnological applications (Adegboye and Babalola, 2012). In conclusion, the findings of this study are poised to make significant contributions to both academic knowledge and practical applications in combating antibiotic resistance, thereby addressing a critical global health challenge and improving overall quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ecology, Diversity and Classification of *Actinomycetes*

Actinobacteria are saprophytic, filamentous, gram positive, free living bacteria which produce economically important antibiotics (Valli *et al*., 2012). *Actinomycetes* consist of more features found in bacteria, and have been grouped into Kingdom *Bacteria* and Phylum *Actinobacteria* (Chaudhary *et al*., 2013). The phylum comprises of six classes including: *Acidimicrobiia*, *Thermoleophilia*, *Coriobacteriia*, *Rubrobacteria*, *Actinobacteria* and *Nitriliruptoria* (Gao and Gupta, 2012). The class *Actinobacteria* consist of 16 orders, including formerly proposed orders, *Bifidobacteriales* and *Actinomycetales*. A unique taxonomic trait of *Actinobacteria* is the guanine-cytosine content in the genome, which is above 55% and majority are filamentous producing mycelia (Al-Dhabi *et al*., 2016). Because of their ability to form branching filamentous mycelia, *Actinomycetes* were mistakenly classified as fungi for several years (Adegboye and Babalola, 2012). Currently, *Actinomycetes* are classified into 10 sub-orders, over 30 families and more than 160 genera (Chavan *et al*., 2013). Ecologically, they are adapted to a wide range of environments including salty and fresh water, soils and air (Barka *et al*., 2016). Majority are found in soils, especially rich organic and alkaline soils, where they make up important part of the soil microbial population, marine water and fresh water environments (Barka *et al*., 2016). *Actinomyces* are renowned for their ability to produce a wide range of antibiotics, which has been extensively studied in laboratory settings. However, the quest for novel antibiotics necessitates a broader approach, including the exploration of these bacteria in their natural environments. Field studies play a crucial role in this context for several reasons. Natural environments harbor a vast diversity of microbial life, including unique strains of *Actinomycetes* that are not represented in laboratory cultures. This biodiversity is a vital source of novel genetic and metabolic traits that can lead to the discovery of new antibiotics (Tiwari and Gupta, 2012). As much as laboratory cultivation of *Actinomyces* is essential for controlled studies and industrial production of antibiotics, field studies are indispensable for expanding the scope of antibiotic discovery and understanding the full potential of these remarkable bacteria (Hug *et al.,* 2018)

An elaborate knowledge on the diversity and distribution of *Actinomycetes* could provide insight into microbial ecology and direct the discovery of unique bio-activities such as novel antibiotics (Beattie *et al*., 2011; Hill *et al*., 2011). Research on microbial diversity involves focus on discovery of unknown microbes of biotechnological value, new methods needed to culture previously uncultivable organisms. Research on microbial diversity involves focus on discovery of unknown microbes of biotechnological value, new methods needed to culture previously uncultivable organisms (Srivastava, 2019). This could address the gap in basic understanding of how microbial diversity originates and consequently tackles the need for preserving newly discovered fastidious organisms. The untapped diversity of *Actinomycetes* is an important resource for novel bioactive natural compounds and gene clusters of value to biotechnology (Tiwari and Gupta, 2012). Metagenomics approaches which are currently used in the study of microbial diversity favors recognition of microbes at the DNA or RNA level without cultivating them on selective media (Martínez *et al*., 2017). Culture independent methods can only be used as pre-screening techniques to known diverse genera as well as the presence of new species within each environment, exploitation and realization of their ability to produce new compound in biotechnological field need both isolation and cultivation (Hug *et al.*,2018). Recent changes in Lake Victoria resulting from anthropogenic pollution, increased algal blooms, emergent water hyacinth and rapid siltation have not been accompanied by studies on *Actinomycetes*.

2.2 Effect of Growth Media on Cultivation of *Actinomycetes*

Cultivation of *Actinomycetes* from their natural environments require specialized conditions, because of slow growth rates. Important factors in the process of isolation include identification of the appropriate selective medium, conditions for culturing, and identification of candidate colonies for downstream screening. Culturing *Actinomycetes* in multiple media is important for identification of media formulations that promote robust growth and secondary metabolite production in different strains.This information is valuable for selecting suitable culture conditions for downstream applications,such as natural product discovery and bioremediation (EI Karkouri *et al*.,2019).Researchers can assess the effects of media composition on colony morphology, pigment production, spore formation, and other phenotypic traits (Li *et al*., 2016). This comparative approach aids in the taxonomic classification, strain characterization, and functional analysis of *Actinomycetes*, contributing to understanding of their diversity and ecological significance. On the other hand, by culturing them in multiple media with different compositions,

pH levels, and nutrient sources, researchers can optimize growth conditions to support the proliferation of a broader range of *Actinomycete* strains (Shama *et al*.,2014). This approach increases the likelihood of isolating and cultivating diverse *Actinomycetes* from environmental samples and facilitates the study of their ecological roles and biotechnological potential.

One of the most important factors that determines growth of *Actinomycetes* is availability of nutrients. Majority of *Actinomycetes* are able to use a wide range of compounds such as glucose, amino acids, proteins, starch, as their energy source, unlike other bacterial groups that only utilizes simple carbon and nitrogen sources (Bhatti *et al.,* 2017).

Indeed, selecting a suitable media and conditions for growth is vital since one selective media may enrich some populations and not others (Schneegurt, 2012). For instance, the use of media containing nitrogen and carbon sources has a great effect on antibiotic production and growth of *Actinomycetes* (Van der Meij *et al*., 2017). To achieve maximum diversities of *Actinomycetes*, different media with varied nutritional values should be used, since nutrient assimilation differs amongst microorganisms (Zothanpuia *et al*., 2018). Earlier reports suggested that several different combinations of media could be used for isolation of *Actinomycetes* including Yeast Malt Extract Agar, Starch Casein Agar and Glycerol Yeast Extract Agar (Bawazir *et al*., 2018). Clearly, growth media composition is vital for the growth of *Actinomycetes.*Yet little is known about factors influencing *Actinomycete* growth in vitro and development of improved media formulations that support the cultivation and exploitation of *Actinomycetes* from sediments of Winam Gulf in Lake Victoria.

2.2.1 Criteria Used to Choose Media Types

When isolating and cultivating *Actinomycetes* from environmental samples such as sediments from the shores of Winam Gulf, it is essential to select a variety of media that can support the growth of diverse *Actinomycetes* strains. The choice of media types was guided by the following criteria:

Nutritional Diversity

Actinomycetes are known for their diverse metabolic capabilities and can thrive on a wide range of nutrients (Bhatti *et al*., 2017). Using different types of media with varying nutritional compositions increases the likelihood of supporting the growth of diverse *Actinomycetes* strains (Tiwari and Gupta, 2013). This approach helps in capturing a broad spectrum of *Actinomycetes* present in the sediment samples.

Specific Nutrient Requirements

Different *Actinomycetes* species may have specific nutrient requirements. By employing media with distinct nutrient profiles, the study aims to cater for the specific needs of different *Actinomycetes,* ensuring optimal growth conditions for a variety of strains.

Enrichment of *Actinomycetes*

Some media are particularly effective at enriching *Actinomycetes* while suppressing the growth of contaminating bacteria and fungi (Tiwari *et al*., 2021). This selective advantage is crucial for isolating pure cultures of *Actinomycetes* from environmental samples that typically contain mixed microbial populations.

Previous Successful Use

The selected media types have a proven track record in previous studies for isolating and cultivating *Actinomycetes* (Hug *et a*l., 2018). This empirical evidence supports their effectiveness and reliability in promoting the growth of *Actinomycetes.*

Secondary Metabolite Production

Some media, like Soya Casein Agar, were specifically chosen because they promote the production of secondary metabolites, including antibiotics, which are significant for *Actinomycetes* research (Cappuccino and Welsh, 2018).

2.2.2 Types of Media Used

The following media were used to evaluate in vitro growth of *Actinomycetes*. Each medium was chosen based on its specific nutrient composition and suitability for supporting the growth of various *Actinomycetes* strains:

Malt-Yeast Extract Agar (MYE)

Malt Yeast Extract agar contains malt extract and yeast extract, providing a rich mixture of carbohydrates, vitamins, and growth factors. This medium supports the growth of *Actinomycetes* that can utilize maltose and other sugars, as well as those requiring additional vitamins and amino

acids for growth (Cappuccino and Welsh, 2019). It is particularly useful for isolating strains that require complex organic compounds for growth (Chauhan *et al*., 2020).

Peptone B Agar (PBA)

Peptone B agar contains peptones, which are partially hydrolyzed proteins, providing a readily available nitrogen source(Abba,2021). This medium supports the growth of *Actinomycetes* that utilize simple organic nitrogen compounds (Madigan *et al*., 2009).

Soya Casein Agar (SCA)

Soya Casein agar combines soybean meal and casein, offering a rich source of amino acids and peptides. This medium supports the growth of *Actinomycetes* that thrive on proteinaceous substrates and promotes the production of secondary metabolites, including antibiotics. (Cappuccino, 2018)

Yeast Extract Agar

Yeast extract is rich in vitamins, amino acids, and growth factors. However, it may not provide the specific carbon and nitrogen sources preferred by many *Actinomycetes.* This medium was included to assess the growth of strains that might have specific requirements for growth factors and vitamins (Madigan *et al*., 2009). The use of Soya Casein Agar, Malt-Yeast Extract Agar, Peptone B Agar, and Soya Casein Agar ensures a comprehensive approach to cultivating a wide range of *Actinomycetes* from the sediments of Winam Gulf. By providing diverse nutritional environments, these media types increase the chances of isolating novel *Actinomycetes* strains with potential antibacterial activity, addressing the urgent need for new antibiotic sources in the face of rising antibiotic resistance.

2.2.3 Growth Characteristics of *Actinomycetes* Using Low-Cost Media

Actinomycetes, renowned for their ability to produce bioactive compounds, are often cultured using various growth media. This review focuses on studies exploring the growth characteristics of *Actinomycetes* when cultivated on low-cost media, highlighting their efficacy and limitations. low-Cost Media alternatives including Corn steep liquor has been successfully used as a low-cost medium for A*ctinomycete* cultivation, providing essential nutrients and supporting robust growth (Alessandrello *et al*., 2017). Studies have shown that soybean meal is an economical alternative,

promoting growth and enhancing secondary metabolite production in *Actinomycetes (*Mitrović *et al*., 2017). Inaddition utilization of agricultural by-products such as wheat bran and rice bran has proven effective in reducing cultivation costs while supporting adequate growth of *Actinomycetes* (Radhakrishnan *et al*., 2010).

Research comparing low-cost media with traditional formulations indicates comparable growth rates and secondary metabolite yields, demonstrating the feasibility of economical cultivation methods (Berdy, 2005). Optimizing nutrient ratios and growth conditions in low-cost media further enhances the productivity of *Actinomycetes*, offering sustainable alternatives to expensive growth substrates (Kumar *et al.*, 2021).

2.3 Isolation Technique for *Actinomycetes*

The successful isolation of *Actinomycetes* using various techniques has been well-documented in the literature, these methods are crucial for studying *Actinomycetes* that produce antibiotics and other bioactive compounds. For instance, Ay *et al*., (2018) employed a soil dilution method with Starch Casein Agar to isolate thermophilic Streptomyces from environmental samples. Their approach involved mixing approximately 1 gram of soil with 9 ml of 1/4 strength sterilized Ringer's solution, followed by manual shaking to disperse bacteria. Pre-heating the suspension at 55°C for 6 minutes was crucial for enhancing the recovery of thermophilic species. Subsequently, serial dilutions were prepared and spread on Starch Casein Agar plates supplemented with cycloheximide and rifampicin. After incubation at 55°C for 7 days, colony-forming units were counted to quantify the isolated *Streptomyces* strains.

In another study, Singh *et al.,* (2016) conducted a comprehensive investigation to isolate novel *Actinomycete*s strains from various locations in India. They utilized multiple isolation techniques, including Starch Casein Agar, HV agar, and ISP media, to capture a diverse range of *Actinomycetes*. By adjusting their methods to specific environmental conditions and using selective media, they successfully isolated strains with potential antimicrobial applications.

Baoune *et al*., (2018) effectively isolated endophytic Streptomyces spp. from plants grown in petroleum-contaminated soil in southern Algeria using HV agar. Their approach provides a robust example of leveraging specific media to support the growth and isolation of *Actinomycetes* from challenging environments. In their study, approximately 1 gram of plant root material was aseptically placed into 9 ml of sterile Ringer's solution and manually shaken to disperse the endophytic bacteria. The suspension was subjected to serial dilution, and aliquots of the diluted

samples were spread on HV agar plates. HV agar is composed of humic acid, which provides a carbon source, and essential vitamins that support the growth of a broad spectrum of *Actinomycetes.* The plates were incubated at 28-30°C for 7-14 days, allowing sufficient time for the colonies to develop. *Actinomycete* colonies, identified by their distinct chalky and filamentous appearance, were subsequently picked and streaked onto fresh HV agar plates to obtain pure cultures. The study demonstrated that HV agar is particularly suitable for isolating Streptomyces spp. from contaminated environments, as it effectively supports the growth of these bacteria while inhibiting many non-target microorganisms. This method is significant as it highlights the importance of selecting appropriate media to enhance the isolation of *Actinomycetes*, especially from complex and contaminated samples.

Furthermore, Teo *et al*., (2014) demonstrated the effectiveness of Starch-Casein-Nitrate Agar for isolating and cultivating soil *Actinomycetes*. This medium supported the growth of both fast-growing and slow-growing species, making it a versatile choice for researchers studying these bacteria.

Traditional isolation techniques have limitations, as highlighted by Tiwari and Gupta (2013). These methods often favor fast-growing or spore-forming strains, potentially overlooking rare or slow-growing species present in environmental samples. Therefore, ongoing research focuses on refining and developing new techniques to comprehensively sample the diversity of *Actinomycetes* and explore their biotechnological potential. Recent advancements in molecular techniques, such as metagenomics and high-throughput sequencing, offer promising tools for uncovering the hidden diversity of *Actinomycetes* (Charlop-Powers *et al*., 2016). These methods allow for the identification and characterization of *Actinomycetes* directly from environmental samples without the need for cultivation, thus by passing the biases associated with traditional isolation techniques. Additionally, the development of novel selective media and enrichment strategies tailored to specific environmental conditions and *Actinomycete* types may further improve isolation success (Bérdy, 2012). In conclusion, the diverse methodologies employed for isolating *Actinomycetes* underscore the importance of tailored approaches to effectively study and utilize these bioactive compound-producing bacteria in various fields of microbiological research.

2.4 Occurrence and Distribution of *Actinomycetes*

Actinomycetes are found in different habitats and constitute a very diverse group of microorganisms widely spread in natural ecosystems (Saha *et al*., 2012). Most species are free

living, saprophytic bacteria found in soil, water and as plant colonizers (Chamikara, 2016). Nevertheless, some such as *Actinomycetes israelii* are human pathogens (Valour *et al*., 2014). Several factors influence the population and type of *Actinomycetes* in soil. For instance, *Actinomycetes* are found both at thermophilic (40^{o}C) and mesophilic (25-30^{o}C) environments (Dangol, 2022). Another significant environmental factor that determines the activities of *Actinomycetes* and their distribution is pH. Majority grow at optimum pH of about 7.0. Reports by Vasavada *et al*., (2006) established that production of antibiotics by *Actinomycetes* was also influenced by media containing nitrogen and carbon sources, different levels of pH and salinity. Diversity of plant species grown on a particular soil can also affect *Actinomycetes* diversity. This is due to the fact that different plants secrets different metabolites, hence for microorganisms such *Actinomycetes* to survive, they must adapt to the environment (Oskay *et al*; 2004).

2.4.1 Effects of human activities on distribution of *Actinomycetes*

Human activities have a significant impact on the distribution of microorganisms, such as *Actinomycetes*.Human activities can significantly alter the microbial composition of sediments (Khattab *et al*., 2016). By sampling shores with different levels of human impact, the study can assess how these activities influence *Actinomycete* diversity

Practices such as tilling and use of pesticides can have a significant impact on soil microbial communities. A study conducted by Trivedi *et al*., (2018) revealed that increase in agricultural practices led to a decrease in soil microbial diversity and an increase in the prevalence of plant pathogenic microorganisms. Furthermore, the use of pesticides can lead to selective pressure for pesticide-resistant microorganisms. On the other hand, pollution can have a significant influence on microbial communities. A research done by Zhen *et al*., (2019) established that heavy metal pollution led to changes in soil microbial communities and altered microbial metabolic processes. Land use changes such as deforestation can change the physical and chemical properties of soil, resulting in changes in the community of microbes. Melo *et al*., (2021) established that deforestation led to a decrease in soil microbial diversity and abundance. Human activities such as urbanization, agriculture, and pollution have profound impacts on microbial communities in various ecosystems. While the general effects of these activities on microbial diversity are relatively well-documented, specific studies on the impact on *Actinomycetes* remain scarce. *Actinomycetes* are essential for soil health, playing a key role in organic matter decomposition and the production of bioactive compounds (Javed *et al*., 2021). However, the extent to which human activities alter their distribution and diversity is not well understood. Existing research has

primarily focused on broad microbial responses to environmental changes, often overlooking the specific dynamics of *Actinomycetes*. For instance, urbanization can lead to soil compaction and pollution, while agricultural practices might alter soil pH and moisture levels, potentially affecting *Actinomycete* populations. Yet, the precise mechanisms and outcomes of these impacts on *Actinomycetes* have not been thoroughly investigated.

2.4.2 *Actinomycetes* from Terrestrial Habitats

Majority of *Actinomycetes* dwell in the soil and perform vital function of managing the stability of microbes through production antibiotic substances (Chavan *et al*., 2013; Gopinath *et al*., 2013). They degrade complex polymers in the soil including lignin, pectin chitin and cellulose (El-Gammal *et al*., 2014). It has been shown that *Actinomycetes* isolates from soil have antagonistic activities against pathogens that affects plants. A research carried out by Jeffrey, (2008) established that *Actinomycetes* from soils utilized for agriculture exhibited antagonistic effects against some phytopathogens such as *Ralstonia solanacearum* and *Bacillus subtilis*. Two strains of *Actinomycetes* with different antimicrobial activities were isolated in Turkey, one was active against *Erwinia amylovora* and other was active against *Agrobacterium tumefaciens* (Oskay *et al*., 2004). Similarly, in Kenya, a strain of *Actinomycetes* effective against *Pyricularia grisea* was isolated from the soil (Opande *et al*., 2013).

The distribution of terrestrial *Actinomycetes* is highly dependent on the geographical location and physicochemical conditions of the environment, such as temperature, pH, salinity, moisture content, organic matter, aeration and cultivation (Zanane *et al*., 2014). Laboratory culture conditions showed that salinity, pH, and media containing nitrogen and carbon sources affect growth and production of antibiotics by *Actinomycetes* (Vasavada *et al*., 2006). Despite increased knowledge on the ecological requirements of terrestrial *Actinomycetes*, studies have shown that there has been a decrease in discovery of new antibiotics, due to extensive previous screening from soil sources and uneconomic re-discovery of known compounds (Zainal *et al*., 2016). It is therefore necessary to examine *Actinomycetes* with a potential for production of novel antibiotic compounds from unexplored sources like sediments of large fresh water bodies, such as Winam Gulf Lake Victoria.

2.4.3 Aquatic *Actinomycetes*

Actinomycetes are numerous in some marine environments and fresh water (Lam, 2006; Valli *et al*., 2012). This is because *Actinomycetes* display special structural and physiological features that enable them survive in aquatic ecosystem. Unique groups of aquatic *Actinomycetes* are capable of

producing novel secondary metabolites which may be lacking in terrestrial groups (Radajewski *et al*., 2002). The highly dominant species from aquatic environment is *Micromonospora* (Maldonado *et al*., 2005; Eccleston *et al*., 2008). Some genera previously isolated from aquatic environment include *Rhodococcus* and *Amycolatopsis* (Lam, 2006). Unique bioactive metabolites have been discovered from *Actinomycetes* found in aquatic ecosystem which include: abyssomicin from *Verrucosispora* species (Hughes *et al*., 2008; Riedlinger *et al.*, 2004). Lake Victoria, facing significant environmental pressures, including pollution, overfishing, and invasive species, presents a unique opportunity to study the role of *Actinomycetes* in its ecosystem (Kayombo and Jorgensen, 2006). However, the diversity of *Actinomycetes* and their potential bioactive compounds from sediments of Lake Victoria is yet to be established.

2.5 General Characteristics and the life cycle of *Actinomycetes*

Actinomycetes are Gram positive, filamentous bacteria which usually form branching rods or threads with non-septate hyphae, although some may have observable septa (Chamikara, 2016). The body form is a mycelium which may either be branching or non-branching, spiral shaped or straight. The mycelia produce spores which are oval, cylindrical or straight (Chaudhary *et al*., 2013). They exhibit dry, powdery appearance on culture media with dissimilar colors dictated by the media used (Devi *et al*., 2012).

Identification of *Actinomycetes* requires both macroscopic and microscopic examination. Macroscopic characteristics include color, consistency of its shape on different media, the presence or absence of aerial mycelium, extent to which the spore is formed and size (Muthu *et al*., 2013). They form branching aerial and substrate hyphae on media, and majority are immotile but the few ones which are motile are restricted within the flagellated spores (Shama, 2014). Microscopic examination can be used to establish characteristics such as: fragmented or non-fragmented substrate, presence of sclerotia, chain morphology of spores, aerial mycelium and surface ornamentation of spores. Microscopic examination can be used to establish characteristics such as: fragmented or non-fragmented substrate, presence of sclerotia, chain morphology of spores, aerial mycelium and surface ornamentation of spores (Dave, 2022). Aerial mycelium can be green, gray, and red, white or blue (Shama, 2014). A situation where two colors are observed then both should be recorded. In cases where melanoid pigments such as brownish black, greenish brown, or distinct brown are produced, then the strain is grouped as melanoid pigment positive. On the other hand, absence of melanoid pigments is grouped as melanoid pigment negative. Recording of reverse pigments is determined by distinctive colony color on the plate reverse side which can be recorded

as positive or distinctive negative. When the color is pale or yellowish brown, it is noted as negative. In a situation where it produces pigments which are soluble in addition to melanin, the color is recorded as either yellow, orange, blue, violet or green. A strain lacking a pigment will be will be recorded as non-pigment producer or negative for pigment production (Sharma, 2014).

2.5.1 *Actinomycetes* Life Cycle

The life cycle of *Streptomycetes* has been extensively evaluated in comparison to other genera of *Actinomycetes*. Growth cycle of the colony is initiated on the surface of the agar from spore to branched filaments, which form substrate mycelium (Miguélez *et al*.,1999). The substrate mycelium develops into aerial hyphae, which extends aerially. The two mycelia are described as multinucleated. The aerial hyphae can be septated into compartments, which differentiate into spore chains (Miguélez *et al*., 2000).

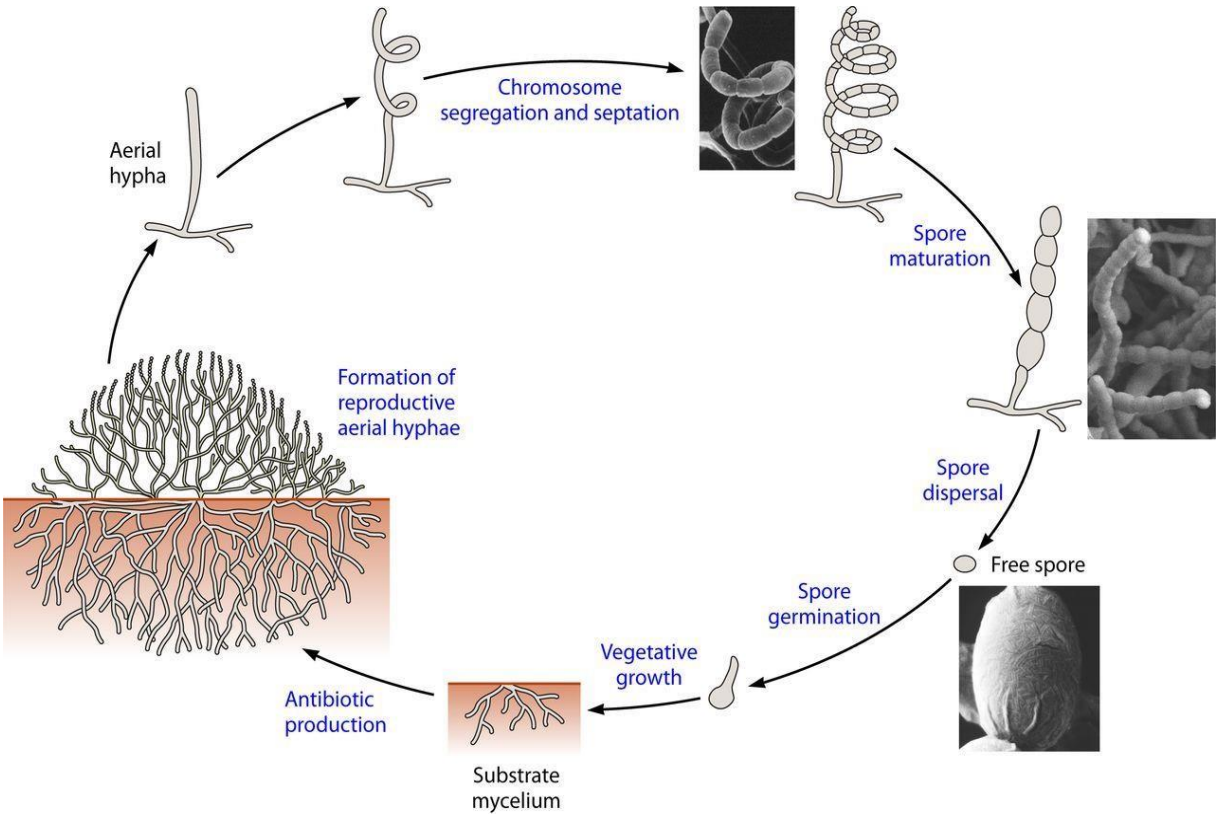

Figure 1. Life cycle of Actinomycetes Source: (Miguelez *et al*., 2000)

Industrial processes which require secondary metabolite production use liquid cultures since metabolites are produced after growth (Martin *et al.,* 2005; Barrios *et al*., 2003). Secondary metabolite production rarely related to growth or organism's reproduction. However, they are for defense against predators of *Actinomycetes* filaments. These metabolites can be harmful to the

producer *Actinomycetes* and their production is directed by genes clustered with those which confer resistance to that particular compound, protecting the organism from self-suicide (Martin *et al.*, 2005).

2.6 Screening of *Actinomycetes* for bioactivity

Actinomycetes secrete antimicrobial compounds that inhibit growth of other microorganisms through various mode of actions including blocking of the synthesis of RNA, cell wall or proteins or through the inhibition of DNA replication (de-Lima *et al*., 2012). Bioactivity spectrum of antimicrobial compounds differs; for instance, some antibiotics can only kill gram-positive but not gram-negative bacteria, while others may kill both (Coates *et al*., 2002). Generally gram-negative bacteria are more resistance to antimicrobial compounds as compared to gram-positive bacteria. The difference is majorly due to their cell wall constituents and functions which could limit the uptake of antimicrobial compounds inside the cells, thus restricting the destructive effect of the compound. Bioactivity spectrum of antimicrobial compounds differs; for instance, some antibiotics can only kill gram-positive but not gram-negative bacteria, while others may kill both (Jubeh *et al*.,2010). Determination of antimicrobial activities of an antimicrobial agent can be done using primary bioassay screening, followed by evaluation of secondary metabolites with potential antimicrobial properties from the selected strains can be evaluated in the secondary screening.

Several bioassay methods could be employed for the initial screening including agar overlay method and perpendicular streak method (Velho-pereira and Kamat 2011). In perpendicular streak method, the *Actinomycete* strain is streaked on agar medium as a single line across the agar followed by incubation for about seven days. The strain will produce antimicrobial compound(s) which will diffuse into the agar. Bacterial test strains are then inoculated as a single streak perpendicular to the *Actinomycete* culture. After further incubation, antimicrobial activity can be observed directly, whereby the growth of test strains along the streaking line nearer to the *Actinomycete* might be inhibited (Velho-pereira and Kamat, 2011). In agar overlay method, the *Actinomycete* strain is spot inoculated onto agar medium. After several days of incubation, another layer of soft agar containing a standardized suspension of the test strain is then added. Further incubation will result in the inhibition area around the *Actinomycete* colony if the antimicrobial compound is present (Gebreyohannes *et al.,* 2013).

In secondary screening, agar diffusion method using either agar-well diffusion or disk diffusion can be employed to evaluate the presence of antimicrobial compounds in the spent fermentation broth or the organic crude extracts of *Actinomycetes* (Balouiri *et al*., 2016). In both methods, a

standardized suspension of test strain from an overnight culture is inoculated onto agar medium. Paper disk impregnated with the crude extract suspension is then placed onto the agar surface. For agar-well method, the crude extract suspension or the spent fermentation broth will be introduced directly into the agar well. The antimicrobial compound will diffuse into the surrounding agar and impede growth of test strain, producing the inhibition diameter around the agar well or the paper disk. Agar disk diffusion method is also used as a standard method to evaluate the susceptibility of bacterial pathogens towards antimicrobial drugs (Jenkins and Schuetz 2012).

Minimum inhibitory concentration (MIC) is a significant assay which can be conducted to evaluate susceptibility of a microbial test strain against an antimicrobial compound (Kowalska-Krochmal *et al*., 2021). For the determination of MIC, dilution method or agar disk diffusion method can be employed. Thus, MIC is the lowest concentration of an antimicrobial compound that can inhibit the "visible growth" of the test microorganism after it was incubated overnight in the presence of the antimicrobial compound (Kowalska-Krochmal *et al*., 2021). Consequently, the lowest fungicidal concentration or lowest bactericidal concentration can be performed to determine the nature of the growth inhibition caused by the antimicrobial compound, as to whether it was bactericidal or fungicidal (Balouiri *et al*., 2016).

2.7 Bioactive Metabolites from *Actinomycetes*

Actinomycetes are the most significant prokaryotes biotechnologically and economically (Deepa *et al*., 2013; Mohanraj and Sekar, 2013). They produce secondary metabolites which have diverse chemical features. Major classes of antibiotics include anthracyclines, glycopeptides, tetracyclines, polyenes aminoglycosides and ß-lactams (Adegboye and Babalola, 2013). The metabolites have varying mechanism of actions such as RNA synthesis, cell wall synthesis inhibition, DNA replication, protein synthesis, and effects on important cellular functions (Solecka *et al*., 2012: de Lima *et al*.,2012). *Actinomycetes* produce secondary metabolites with various invaluable capabilities including; antibiotics, enzymes, tumor-treating agents, immune inhibitors, nutritional materials, cosmetics and vitamins (Jeya *et al*., 2013). Diversity of *Actinomycetes* metabolite bioactivity is attributed to the elaborate genome, with transcription factors that control gene expression, and thereby respond to required needs (Barka *et al*., 2016). Production of antibiotics is controlled by genes that impart full expression as influenced by biotic and abiotic factors (Ponmurugan and Nithya, 2008). A new species of known as *Streptomyces bangladeshensis* capable of producing 2-ethylhexyl was discovered from soil samples in Bangladesh (Al-Bari *et al*., 2005). *Actinomycin* compound was isolated from a new strain of

Streptomyces parvulus found in soils collected at Rajshahi in Bangladesh (Arifuzzaman *et al*., 2010).

Before the metabolites from *Actinomycetes* are chemically processed, their bioactivity need to be tested against pathogenic microorganisms. For instance, *Streptomyces sannanensis* isolated from various states in Southern India were capable of secreting antibiotics against certain Gram positive bacteria which included: *Bacillus subtilis* and *Staphylococcus aureus* (Ponmurugan and Nithya, 2008). The enzymatic activity of these antibacterial compounds of *Actinomycetes* isolated from the fresh water systems revealed that they possessed amylase, cellulase, protease and urease activity but lacked chitinase activity (Gunda *et al*., 2012). Other economically important compounds produced by *Actinomycetes* includes: vitamins, enzymes and immune modulators in addition to antibiotics (Moncheva, 2002). An extracellular protease producing *Actinomycete, Streptomyces nogalator* strain was reported to be efficient in depilation of goatskin (Mitra, 2005). *Actinomycetes* also produce secondary metabolites such as lactones, alimphatic alcohols, ketones, esters, thioesters, biogenic sulphides, isoprenoids and furanones lactones which are important in pharmaceutical and chemical industry (Zaitlin and Watson, 2006).

2.7.1 Future Prospects of Metabolites from *Actinomycetes*

Secondary metabolites are secreted by microbes which possess potent and sometimes unique bioactivity for relatively untapped potential with high antibiotics research prospects in the future (Berdy, 2012). Common *Actinomycetes* that are potential drug sources are not cultivable and are not easily accessible for novel antibiotic production. Very little percentage of the 10^{12} soil *Actinomycetes* has been screened (Baltz, 2008). One to three percent of antibiotics producing *Streptomycete* has been discovered and the rest requires screening, selection and enhancement (Goodfellow, 2010). Chemical diversity of bioactive compounds, particularly from rare *Actinomycetes*, is promising. However, discovery of bioactive *Actinomycetes* taxa calls for an elaborate thinking of their correct diversity and eco-physiology through which target directed isolation strategies can be carried out (Starch *et al*., 2004). Great challenges such as antibiotic resistance and environmental pollution make income from microorganisms to be of interest in providing alternative solutions.

Bioactive compounds produced by *Actinomycetes* show an antibacterial and antimicrobial activity against different pathogens and multi-drug resistant pathogens such as Methicillin resistant *Staphylococcus aureus, Shigella dysenteriae, Klebsiella* sp, *Escherichia coli, Pseudomonas aeruginosa,* Vancomycin resistant *Enterococci* and many more (Selvameenal *et al*., 2009; Singh

et al., 2012). Majority of death cases are reported annually as a result of bacterial infections and other infectious diseases. The future prospects of metabolites from *Actinomycetes* are vast and promising, with potential applications spanning pharmaceuticals, industry, agriculture, and environmental management. Continued research, leveraging advanced technologies and interdisciplinary approaches, is essential to unlock the full potential of these remarkable microorganisms and their metabolites. Yet cultivable *Actinomycetes* from sediments of Lake Victoria that may have prospects for production of elite bioactive secondary metabolites has not been fully established.

2.8 Economic Importance of *Actinomycetes*

Economically and biotechnologically, *Actinomycetes* are used in the production of secondary metabolites which are used to produce important substances, which range from antibacterial to viral, anticancer agents, enzymes and vitamins. These metabolites are used in the agricultural sector to produce pesticides and herbicides (Manivasagan *et al*., 2013). Biotechnologically, the secondary metabolites generate important products such as antibiotics to anticancer agents, enzymes and vitamins (Selim *et al.*, 2021).

2.8.1 Medical Importance of *Actinomycetes* Compounds

Actinomycetes play a major role in human health, due to their capacity to provide major sources of antibiotics (Jose and Jha, 2016). Approximately 70% of metabolites produced by *Actinobacteria* exhibit antimicrobial activities (Bérdy, 2012), and 64% of the identified natural product antibiotic classes are produced by filamentous *Actinomycetes* (Hutchings *et al*., 2019).They produce Several clinically used antibiotic classes, such as ansamycins ,beta-lactams glycopeptides aminoglycosides macrolides and tetracyclines.They produce Several clinically used antibiotic classes, such as ansamycins beta-lactams glycopeptides aminoglycosides, macrolides; and tetracyclines (Adegboye and Babalola, 2013). Consequently, they are also capable of producing important antibiotics with different mode of action against pathogens such as; phosphomycin from *Streptomyces fradiae* and streptomycin from *Streptomyces griseus* (Manteca and Sanchez, 2010). Some target bacterial ribosomes and hence are used in the treatment of respiratory infections for example both erythromycin and tetracycline are active against Legionnaires' disease (Sharma, 2014).

2.8.2 Importance of *Actinomycete*s in Agriculture

Actinomycetes are important constituents of most soils, because they are saprophytic in nature, and produce a variety of extracellular hydrolytic enzymes which are able to degrade animal and plant

polymers, including chitin, lignin, cellulose, and many other organic substances (Eisenlord and Zak, 2010). This ability to infiltrate and solubilize polymers enables them to continue in the microbial succession (Kandasamy *et al*., 2012). Their unique lifestyle enables them to play important role of cycling organic matter in the soil ecosystem, biological buffering of soils and biological control of soil environments through nitrogen fixation and degradation of high molecular weight compounds like hydrocarbons in the soils which are polluted (Chaudhary *et al*.,2013). Several studies that have been conducted revealed that *Actinomycetes* are able to degrade a range of compounds which included lignocelluloses and a variety of xenobiotic compounds (Atiwesh *et al*., 2022). The main enzymes involved in degradation of lignin substructures have been identified (De Gonzalo *et al*., 2016). *Streptomyces viridosporus, Thermomonospora mesophil* and *Streptomyces badius* have all been evidenced to degrade lignin under diverse environmental conditions and the final products of biochemical reactions have been investigated (Borgmeyer and Crawford, 1985; Giroux *et al*., 1988; Godden *et al*., 1992). It has been established that *Streptomyces setonii* has also demonstrated catabolism of vanillic acid and there is additional evidence that lignin-related compounds can be degraded by rhodococcus and norcardia as also demonstrated catabolism of vanillic acid and there is additional evidence that lignin-related compounds can be degraded by rhodococcus and norcardia (Goodfellow and Williams, 1983). The studies established that *Actinobacteria* like arthrobacter was activated by increase of hydrocarbons in the soil and *Rhodococcus* species and *Norcardia steroids* were common in soils polluted with petroleum (Ivshina *et al*., 2017). A study that was carried out to examine the ability of *Actinomycetes* which included *Rhodococcus* species, *Norcardia* species, *Gordonia* species and *Micromonospora* species and some bacteria such as *Micrococcuss* and *Sporosarcina* species were capable of degrading motor cycle spent oil (Idemudia *et al*., 2014). The ability of *Actinomycetes* to degrade hydrocarbon was however higher than that of bacteria. In other studies, *Rhodococcus, Corynebacterium* and *Microbacterium* strains were present in stimulated sludge from dairy (Goodfellow and Williams, 1983). Studies have shown that *Actinomycetes* are responsible for the characteristic earthy smell, this is because they are able to produce a substance known as *geosmin* (Anuar *et al*., 2017). Previous evidence revealed that antibiotics isolated from different regions were capable of hindering plant pathogens for example; *Actinomycetes* isolated from turkey's farming soil had the ability to inhibit *Agrobacterium tumefaciens*, a bacterium that cause crown gall disease and *Erwinia amylovora*, a causative agent of fire blight apple (Oskay *et al*., 2004). In Kenya, *Actinomycetes* isolated from the soil had the ability to inhibit *Phytophthora infestans* which causes tomato late blight disease (Mutitu *et al*., 2008). The surge in soil pollution

as a result of use of herbicides and pesticides has seen majority of microorganisms being used as biological control agents. *Actinomycetes play* a significant role in biological control of insects through production of bioactive compounds against insects. Insecticidal active compounds against *Musca domestica* has been confirmed (Selimet *et al*., 2021). *Streptomyces* species produced metabolites that had insecticidal activity against second instar larvae of *Sitophilus oryzae* which is a rice weevil that infects most crops (Haque *et al*., 2013).

2.8.3 Importance of *Actinomycetes* in Bioremediation

Actinomycetes are important in composting and vermicomposting since they decompose starch, cellulose and proteins. Certain *Actinomycetes* produce enzymes which break down lignocellulose components such as lignin, cellulose and hemicellulose (Limaye *et al*., 2017). The use of microbes, which are capable of degrading hemicellulose and cellulose, plays a significant role because of high percentage of these cellulose and hemicellulose contents in plant biomass (Jeffrey, 2007). A study by Lin *et al.,* (2011) established that a strain of *Streptomyces parvulus*, isolated from waste-water sludge could degrade a pyrethroid based insecticide named cypermethrin. Polti *et al*., (2007) argued that if bioremediation of heavy metals and other organic compounds was to be considered then *Actinomycetes* were relevant, owing to the fact that they constitute a prevalent microbial component in most soil biota, attributed to their metabolic diversity, characteristics of their growth, mycelial form and the ability to rapidly colonize selective substrate.

2.9 *Actinomycetes* Identification

Identification of *Actinomycetes* considers their morphological, physiological, biochemical and molecular characters.

2.9.1 Morphological Techniques

Morphology of *Actinomycetes* on specific media may provide a hint on its identification. These morphological features are used to characterize *Actinomycete* genera based on formation of zoospores or sporangia or occurrence of spores on the substrate mycelium (Atta *et al*., 2011). The substrate mycelium color may range from orange to yellow or brown, while aerial mycelium can also vary from creamy, white, chalky, powdery, grey to pinkish and brown (Salim *et al*., 2017). It has been established that they form rings which are concentric when they age up (Devanshi *et al*., 2021). Certain strains produce melanoid pigments which vary from brown, brown black, light-green, or distinct brown. Apart from production of melanoid pigments there are some *Actinomycetes* which produce soluble pigments which vary from orange, yellow, blue, red and green while some strains are capable of producing a pigment on the reverse side (Sharma, 2014).

2.9.2 Biochemical Methods

Biochemical tests are used for *Actinomycetes* strain comparison and differentiation. These include; test for casein hydrolysis which is demonstrated by clear zones around the colonies; utilization of citrate test to determine ability to metabolize citrate as a carbon source Harold, (2002), urea hydrolysis and catalase tests (Cappuccino and Sherman,2002). Using the morphological characteristics and biochemical tests results, it is possible to identify the isolates using the reference strains provided in the Bergey's Manual of Determinative Bacteriology.

2.9.3 Molecular Characterization

Molecular characterization involves the study of nucleic acids or their gene products to establish the relationship between strains or species (Clarridge, 2004). Molecular analysis includes identification and classification whose origin is from past studies of nucleic acid hybridization. The RNA-based genes are significant in analysis of microorganisms for the purposes of taxonomy. For instance, characterization of *Actinomycetes* has been done through sequencing the gene for 16S rRNA (Byrne, 2020). The gene 16S rRNA is important in protein synthesis hence is found in all *Actinomycetes* and are useful indicators of relationships (Gentry *et al*., 2006). This gene is ideal for evolutionary analysis since the capacity of its segments to undergo lateral transfer among organisms is extremely minimal, and it contains conserved regions (homeobox), moderately variable and highly variable nucleotides (Gentry *et al*., 2006). The conserved genes are important in offering primer sites for PCR and convenient 16S rRNA hybridization targets (Letowski *et al*., 2004). Phylogenetic analyses of *Actinomycetes* is based on sequences of the 16S rRNA gene, which consists of 1542 bp since the gene is highly conserved among the *Bacteriaceae* (Clarridge, 2004; Sacchi *et al*., 2002). Genomic DNA can be extracted from an *Actinomycete* isolate, and the 16S rRNA gene amplified by polymerase chain reaction using primers that target the gene sequence (Jeffrey, 2008). Purification and sequencing of the products are done using a DNA sequencer which provides the base order of arrangement within the sample length (Sacchi *et al*., 2002). The sequences are then compared to those in the Gen Bank database at the website of the National Centre for Biotechnology Information, using phylogenetic analysis procedures. While there is existing literature on the characterization of bioactive *Actinomycetes* focusing mainly on their morphological, physiological, and biochemical properties, there remains a gap in the comprehensive understanding of their genomic and metabolic profiles.

2.9.4 Modern Identification Methods for *Actinomycetes:* Strengths and Weaknesses

Actinomycetes are valuable sources of bioactive compounds, necessitating accurate identification methods that balance strengths and weaknesses across various techniques. This review synthesizes current literature on modern identification methods for *Actinomycetes*, emphasizing their respective advantages and limitations.

Cross-Inoculation: Cross-inoculation provides functional insights into microbial interactions but lacks taxonomic specificity (Berdy, 2005).

Serology: Serological methods offer specificity but are limited by antibody availability and cross-reactivity issues (Waksman, 1961).

Antibiotic Resistance Profiling: Antibiotic resistance profiling is clinically relevant but does not provide species-level identification (Wright, 2012).

DNA-DNA Hybridization: DNA-DNA hybridization offers high specificity but is labor-intensive and requires specialized equipment (Kolpashchikov, 2019) 16S rRNA Gene Sequencing: 16S rRNA gene sequencing provides precise identification at the genus and species levels but is costly and time-consuming (Clarridge, 2004).

Whole Genome Sequencing (WGS): WGS offers comprehensive genetic data and high resolution but is expensive and requires advanced bioinformatics (Schwarzeet al., 2018).

MALDI-T of Mass Spectrometry: MALDI-T of mass spectrometry provides rapid results and high throughput but is database-dependent and may lack resolution for closely related species (Seng *et al*., 2009). Metagenomics: Metagenomics allows for comprehensive analysis of microbial communities but requires complex data analysis and is costly (Handelsman *et al*., 1998).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

Sediments used for isolation of *Actinomycetes* were obtained from the shores of Winam Gulf, Lake Victoria. Lake Victoria has a surface area of 68,860 km^2, a catchment area of 193,000 km^2, altitude of 1134 m above sea level and approximately 400 km long by 250 km wide. It is shared by the three East African countries and Kenya occupies about 4,128 km^2 (Ntiba *et al*., 2001). The main rivers flowing into the lake include: Nzoia, Kuja, Yala, Nyando, Sondu Miriu, North Awach, South Awach, and Sio from the Kenyan side (Werimo *et al* 2006). Winam Gulf connects to the main lake via Rusinga channel South of Uyoma point and extends as a shallow (2-4 m) indented bay Eastwards to Kisumu (Gikuma-Njuru *et al.*, 2013). The shoreline is approximately 500 km long with flat sandy and or muddy areas, the latter being predominant in the sheltered bays. Winam Gulf has a tropical climate, and experiences four climatic changes which include; the long rains, short rains, long dry and the short dry season annually (Anyah *et al*., 2022). The Gulf has an annual temperature range of 18.6-25ºC and average annual rainfall of 886-2609 mm as captured by morphometric data of Lake Victoria between 1950 and 2000 (Kayombo and Jorgensen, 2006). The high rains in the region are associated with floods and storm waters which washes pollutants form non-point sources into the gulf. The specific sites of sediment collection were Mbita, Luanda Kotieno, Kunya beach, Kendu bay, Usenge, Luanda Kotieno, Homa bay and Kisumu (Fig 2).

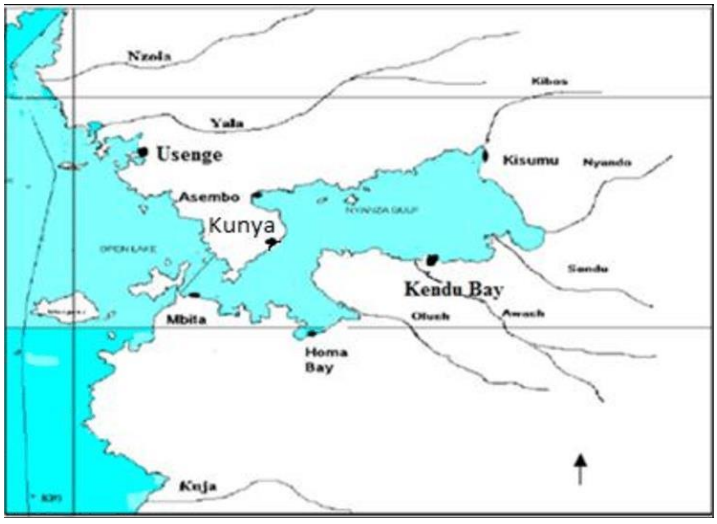

Figure 2. A map of eight sampling points along Winam Gulf shores, Lake Victoria

3.1.1 Description of sampling sites

The sites were selected based on human activities around Winam Gulf of Lake Victoria.

3.1.2 Asembo Bay beach

The geographical coordinates of Asembo Bay are approximately 0.2167°S, 34.6667° E. The shore consists of coarse sand, pebbles and a lot of vegetation, particularly water hyacinth. Little human activities take place which includes little fishing and sand harvesting.

3.1.3 Mbita Beach

Mbita beach is situated on the eastern shores of Lake Victoria, in western Kenya. The beach is situated in the town of Mbita, which is the headquarters of of Suba sub-county, and is surrounded by green vegetation. The geographical coordinates of Mbita beach are approximately 0.4283°S latitude and 34.1989°E. The beach is sandy and the human activities are fishing and motor boat transport (Wanyua *et al*., 2013).

3.1.4 Kunya Beach

Kunya beach is located on the shores of Lake Victoria, in western Kenya. The geographic coordinates of Kunya Beach along Lake Victoria is approximately 0.2443° S, 34.2382° E. The beach is sandy and has a lot of vegetation-water hyacinth. Human activities are limited with little fishing taking place

3.1.5 Luanda Kotieno Beach

Luanda Kotieno beach is located on the shores of Lake Victoria, in western Kenya. The geographical coordinates of Luanda Kotieno beach is approximately 0.0282° S and 34.2511° E. The beach is sandy and rocky and has little floating vegetation. Motor-boat transport is predominantly used, other human activities also includes fishing and farming around the beach.

3.1.6 Usenge Beach

Usenge Beach is located on the northern shores of Lake Victoria, in western Kenya. The geographical coordinates of Usenge Beach are approximately 0.1667° S latitude and 33.9833° E longitude. It is one of the largest fish landing beaches in the region, with about 1000 traders (John Okello, personal communication, 2020). The beach is sandy and attracts tourists due to its windy and cool breezes. Human activities include fishing, hotel industry services, and motorboat transport.

3.1.7 Kisumu Bay

Kisumu Bay is located in the capital city of Kisumu County with an approximate urban and rural population of about 397,957 and 714,668 respectively; totaling 1,155,574 as per the Kenyan Population and Housing Census, (2019). The geographical coordinates of Kisumu Bay are approximately Kisumu bay is approximately 00º05.212″S, 034º44.969'E. The city is a hub of trade and transportation besides being a chief terminus for food processing industries. The region experiences tropical rainforest climatic conditions with an average of 22.9ºC and two rainy seasons. Increased human population and the growth of the city have resulted in pressure on the sewerage and sanitation facilities. The frequent rains experienced in the low-lying area also contribute to storm water pollution. Kisumu Bay receives inputs from the rivers Auji and Nyamasaria which converge and flow via the Dunga village slums through the hippo point, located to the southwest of the city. The streams flow via the papyrus swamps into the lake. River Kisat is smaller and originates from the swamp and flows via Kondele, the Car wash, and into the lake. The river is adjacent to a fish processing plant called Peche food factory and the Nyanza golf club. Kisian River flows into the bay through agricultural settlements and therefore is affected by storm waters from both point and non-point sources, hence the input of fertilizers and pesticides

3.1.8 Kendu Bay

Kendu Bay is the headquarters of the North Karachuonyo sub-county in Homa Bay County along Katito-Homabay road. It is located 30 Km southwest of Homabay and 40 Km of Kisumu City. The town has a population of approximately 37,893 (Kenya Population and Housing Census, 2019). The geographical coordinates of Kendu Bay are approximately 0.3510° S latitude and 34.6554° E longitude. The main human activities are fishing and boat transport.

3.2 Sampling Procedures

3.2.1 Sampling Design

Random selection of eight catchment sites along the shores of Lake Victoria (Fig. 2) was done to evenly represent Winam Gulf of Lake Victoria. The sites were selected based on human activities around Lake Victoria the activities included: Agricultural runoff, urban waste discharge, fishing activities, tourism and recreation, industrial discharge, and domestic waste disposal. At each site, systemic random sampling was used to identify 3 points for sediment sampling (1 m in-shore) from a random starting point with 10 m intervals between sampling points according to Chaudhary *et al*., (2013).

3.2.2 Collection of Samples

A total of 24 samples of soil sediments were collected using a sterilized spatula at a depth of about 15 cm. According to Chaudhary *et al*., (2013), *Actinomycetes* are abundant at a depth between 11 cm -16 cm and their population progressively decreases with increase in depth. The collected sediments samples were transferred to 500 ml wide mouth sterilized bottles within cooler boxes. All samples were labeled as per sampling site and transported to JOOUST Botany laboratory for storage under refrigeration at 4^{o}C for further analysis. Sample collection was done in April 2020.

3.3.1 Pretreatment of Samples

The soil sediment samples were separately air dried for seven days at room temperature, to eradicate the Gram negative bacteria (Vidyasr, 2015). This was followed by heat treatment in a hot air oven at a temperature of 121^{o}C for fifteen minutes to inhibit growth of other bacterial flora (Ayisa *et al.*, 2017).

3.3.2 Isolation of *Actinomycetes* from Sediment Samples

Isolates of *Actinomycetes* were obtained using serial dilution method (Gebreyohannes *et al*., 2013). The stock solution was prepared with 1 g of soil diluted in 9 mL of sterile distilled water and shaking was done using vortex mixer for about two minutes. About 1 mL from the stock solution was used to prepare a dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. About 0.1 mL of the mixture from 10^{-3}, 10^{-4} and 10^{-5} was spread on plates containing four media which included: Soya Casein Agar, Peptone B agar, Yeast Malt Extract Agar and Yeast Extract Agar. Each media was supplemented with Fluconazole 25 µg/ml to inhibit fungal growth and ciprofloxacin 5 µg/mL to suppress bacterial growth (Rao *et al*., 2012). The different media were used to evaluate the morphological diversity of *Actinomycetes* strains and to establish the best media for *Actinomycetes* growth. Incubation of plates was carried out aerobically at a temperature 28^{o}C for 10 days, after which growth was measured in millimeters and pure colonies were sub-cultured on Yeast-Malt Extract Agar and refrigerated at 4^{o}C.

3.4 Screening for Antibacterial Activity

Primary screening of pure isolate was done using Mueller Hinton Agar (Sapkota *et al*., 2020). The *Actinomycete* isolates were spot inoculated at the center of Muller Hinton Agar plates and incubation done for about one week at a temperature of 28^{o}C (Kizhakedathil ***et al***.,2018). Individual plates were flooded with an overnight culture of the various bacteria which included: *Staphylococcus species, Shigella* species, *Streptoccocus* species, *Xanthomonas* species *and Escherichia coli* followed by incubation at 37^{o}C for about 24-48 hours after which inhibition zones

around the colonies were measured. The antibacterial activity of *Actinomycetes* was assessed by flooding individual plates containing *Actinomycetes* colonies with a suspension of test organisms. After incubation, zones of inhibition around the colonies were measured to determine antibacterial efficacy (Jones *et al*., 2021).

Control experiments involved the use of test organisms without *Actinomycetes*. Primary screening was done at a BSL3 laboratories at Kenya Medical Research Institute, Kisian in Kisumu County.

3.5 Pathogenic Strains

The isolates were screened for antibacterial activities against selected strains of bacteria which are normally resistant to drugs, they included: *Staphylococcus* species, *Streptococcus* species, *Shigella sp.*, *Xanthomonas* species and *Escherichia coli*. All the pathogenic isolates were provided by Kenya Medical Research Institute in Kisian found in Kisumu County.

3.6 Morphological, Biochemical and Molecular Characterization

3.6.1 Macroscopic Morphology

Isolates which showed inhibitory activity against the pathogens were sub-cultured on Yeast Extract Malt Extract Agar plates and incubation done at 28^{o}C for 10 days, so as to achieve a good growth. The surface color of mycelium, reverse mycelia color and colony morphology were observed and recorded.

3.6.2 Microscopic Morphology and Gram Stain Test

Pure *Actinomycetes* cultures were placed on clean slides, fixed using heat and placed on a staining rack. Crystal violet was spread on the slides and rinsed off using distilled water. Iodine solution was flooded and the smears left to stand for 1 min, then rinsed with distilled water. Decolorization followed using acetone for five seconds, thereafter rinsing process followed immediately using distilled water so as to avoid excess decolorization. The counterstain safranin was flooded and then rinsed using distilled water. An absorbent paper was used to dry the smears, which were then viewed under a light-microscope at oil-immersion (x400) (Cappuccino and Sherman, 2002).

3.6.3 Biochemical Characterization

The pure isolates were grown on Yeast Extract Malt Agar plates at 28^{o}C for 7 days and a colony was picked and put on a sterile glass slide, with a drop of hydrogen peroxide (Cappuccino and Sherman, 2002). Gas bubbles production showed a positive result.

3.6.4 Molecular Characterization

3.6.4.1 DNA Extraction

Extraction of genomic DNA was done using ZYMO Quick-DNA Fungal/Bacterial Microprep Kit (Catalogue D6007 - Zymo Research, South Africa) according to the manufacturers' instructions (Apendix III).The concentration and purity of extracted DNA was estimated using a Nanodrop™ Lite Spectrophotometer (Model UV-1800 Shmadzu) at 260-280 nm,followed by normalization of the concentration at 50 ng/µl. Molecular weight was determined against the ladder on 0.8% (w/v) agarose gel according to Kuhn *et al*.,(2018)(Appendix IV)

3.6.4.2 Amplification and sequencing

Polymerase chain reaction (PCR) amplification of the 16S rRNA gene region was performed in a programmable thermo-cycler (C1000-BioRad, USA) using the PCR conditions described by Vellinga *et al*., (2003). The primers used for the 16S rRNA gene were adopted from White *et al*., (1990) and included; forward primer 27F (AGA GTT TGA TCM TGG CTC AG) and the reverse primer 1492R (GGT TAC CTT GTT ACG ACG ACT T) using the amplification protocols described by Schoch, (2012). The PCR was performed in a 25-µl reaction mixture comprising of 2.5 µl input of DNA, double-distilled water (16.25 µl), 2.5 µl of USB 10× buffer with $MgCl_2$ (10 mM; 1 µl of USB $MgCl_2$ (25 mM), 0.5 µl of deoxynucleoside triphosphate (dNTP) mixture of 10 mM each; 0.25 µl AmpliTaq polymerase (5 U/µl; Applied Biosystems, Carlsbad, CA), 0.5 µl of Hotstart-IT DNA Fidelitaq polymerase; Affymetrix), and 1 µl (5 µM) of each primer (Usyk *et al*, 2017). The PCR amplification procedure was initiated at 94°C (4 min), 35 cycles of denaturation at 94°C (1 min), annealing at 56°C (1 min) and extension at 72°C (1 min), with final extension at 72°C (10 min). The PCR amplicons were purified using a Thermo Scientific®GeneJET Purification Kit (EU, Lithuania) according to the manufacturer's specifications. The PCR amplicons were then submitted to Macrogen Europe BV, in Netherlands for Sanger sequencing using the same 16S rRNA primers (https://www.macrogen-europe.com/services). Sequences were cleaned using geneous version to remove chimeras (Chen *et al*., 2016).

3.7 Phylogenetic analysis of the 16S rRNA gene sequence

Contigs, which are consensus sequence of both the reverse and forward sequence was generated using the BioEdit software aligner (Hall, 1999). The 16S rRNA sequences generated were compared to sequences in the National Centre for Biotechnology Information (NCBI) GenBank database using the Basic Alignment Search Tool (BLAST) (Altschul *et al.*, 1990). Phylogenetic

and molecular evolutionary analysis was then conducted using MEGA version 6.0 (Tamura *et al.*, 2013). The sequences were aligned using the CLUSTAL W program against the nearest neighbors and the evolutionary history was inferred using the Neighbor-Joining method (Tiwari *et al*., 2015). The evolutionary distances were computed using Jukes-Cantor method and were in the units of the number of base substitutions per site. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Patrick *et al*., 2018).

3.8 Data Analysis

Morphological and biochemical phenotypes of *Actinomycetes* colonies were directly presented using pictograms, tables and descriptive data. Numerical data on *in-vitro* cultural growth and antibacterial activity were analyzed on SAS version 21 by Analysis of Variance (ANOVA) at 95% confidence level and means that were significant separated by Least Significant Difference ($p \leq 0.05$). Molecular data analysis was analyzed using BioEdit software aligner and assembled sequences were transferred to MEGA Version 6.0 and aligned using CLUSTAL W according to Tamura *et al*., (2013). Individual consensus sequences of the 16SrRNA gene regions were used to evaluate closely related sequences at the NCBI GenBank (www.ncbi.blast.lnm.nih.gov)using Basic Local Alignment Search Tool (BLAST Query).

CHAPTER FOUR
RESULTS

4.1 Evaluation of *In-vitro* Growth of *Actinomycetes*

4.1.2 Effect of Media on *Actinomycetes* Colony Growth

A total of 24 sediment samples were cultured in-vitro on four different media (Yeast Malt Extract Agar, Soya Casein Agar, Peptone B Agar and Yeast Extract Agar). Only 16 sediment samples successfully developed *Actinomycetes* colonies in culture, with varying growth rates (mycelia length in millimeters). Yeast Malt Extract Agar produced the highest mycelia length of 14.32±0.06 mm, followed by Soya Casein Agar with 12.82±0.04 mm, and Peptone B Agar with 10.87±0.01 mm. The least mycelia length of 10.39±0.03 mm was observed on Yeast Extract Agar. There was a significant difference ($p \leq 0.05$) in the growth of *Actinomycetes* isolates cultured on different media after 10 days of incubation (Table 4.1).

Table 4.1 *In-vitro* growth of *Actinomycetes* isolates on different media

Media type	Growth (mm)
Yeast-Malt Extract Agar	14.32±0.06[a]
Soya Casein Agar	12.82±0.04[b]
Peptone B Agar	10.87±0.01[b]
Yeast Extract Agar	10.39±0.03[c]
***p* value**	<.0001
LSD ($p \leq 0.05$)	0.2407
CV (%)	5.3612

Note: Means followed by the same super script letters along the column are not significantly different

4.1.3 Colony Growth of *Actinomycetes* Based on Site of Isolation

After 10 days of incubation, of *Actinomycetes* isolates from different collection sites (Table 4.2). The highest growth was observed in isolate A3 from Asembo-bay with a mycelia length of 14.67±0.06 mm. This was followed by isolate HB3 from Homa Bay, which had a growth of

13.75±0.00 mm. The least growth was observed in isolate L3A from Luanda Kotieno, with a growth rate of 10.20±0.08 mm. There was a significant difference ($p \leq 0.05$) in their growth rate (mycelia length in mm).

Table 4.2 Mycelia growth of *Actinomycetes* mycelia based on site of isolation

Isolate	Site of sample collection	Mycelia Growth (mm)
A3	Asembo Bay	14.67 ± 0.06^{a}
HB3	Homa Bay	13.75 ± 0.00^{b}
L3B	Luanda Kotieno	13.08 ± 0.03^{c}
K3	Kunya Beach	12.83 ± 0.01^{c}
U3	Usenge Beach	12.08 ± 0.04^{d}
KB3	Kendu Bay	11.20 ± 0.01^{e}
MT3	Mbita	10.92 ± 0.00^{ef}
KS3	Kisumu	10.67 ± 0.03^{f}
L3A	Luanda Kotieno	10.20 ± 0.08^{g}
***p*-value**		<.0001
LSD ($p \leq 0.05$)		0.3742
CV (%)		5.3612

NOTE: Means followed by the same super script letters along the column are not significantly different

4.2 Antibacterial Activity of *Actinomycetes* Isolates

The average antibacterial activity of *Actinomycetes* isolates (MT3, K3, KB3, L3B, L3A, U3, KSM3, HB3, and A3) was evaluated against pure isolates of five bacterial test pathogens (*Staphylococcus sp., Streptococcus sp., Xanthomonas sp., Escherichia coli sp., and Shigella sp.*) and recorded in Table 4.3. The *Actinomycetes* showed significant ($p<0.05$) variations in their antibacterial properties (inhibition zones in mm) against the test pathogenic bacteria. Staphylococcus species were the most highly inhibited by *Actinomycetes* isolates, showing the highest inhibition zone of 5.81±0.09 mm. *Streptococcus* species were also highly sensitive, with an inhibition zone of 3.63±0.71 mm. However, there was no significant ($p>0.05$) variation in the inhibition zones observed for *Shigella* (0.70±0.01 mm) and *Escherichia coli* (0.78±0.03 mm) species.

Table 4.3 Antibacterial activity of *Actinomycetes* isolates against specific bacterial pathogens

Pathogen	Inhibition zone (mm)
***Staphylococcus* sp.**	5.81±0.09[a]
***Streptococcus* sp.**	3.63±0.71[b]
***Xanthomonas* sp.**	1.36±1.43[c]
Escherichia coli	0.78±0.03[c]
***Shigella* sp.**	0.70±0.01[c]
Control	0.00±0.00[c]
P value	< 0.0001
$LSD_{(p\leq0.05)}$	1.3611
CV (%)	15.9671

NOTE: Means followed by the same letter superscript are not significantly different ($p\leq0.05$)

Isolate MT3 produced inhibition zone of (5.83±0.02 mm), which was significantly ($p<0.05$) different compared to other *Actinomycetes* isolates (Table 4.4). Inhibition zones of isolate K3 (3.14±0.08 mm), KB3 (2.07±0.01 mm), KSM3 (1.96±0.04 mm), L3B (1.84±0.02 mm), HB3 (1.83±0.03 mm) and A3 (1.79±0.03 mm) were not significantly different ($p>0.05$).

Table 4.4 Antibacterial activity of *Actinomycetes* based on site of isolation

Isolate Identity	Site of Isolation	Zone of Inhibition (mm)
MT3	Mbita	5.83±0.02[a]
K3	Kunya Beach	3.14±0.08[b]
KB3	Kendu Bay	2.07±0.01[b]
KSM3	Kisumu Bay	1.96±0.04[b]
L3A	Luanda Kotieno	1.92±0.01[b]
U3	Usenge Beach	1.86±0.01[b]
L3B	Luanda Kotieno	1.8±0.02[b]
HB3	Homa Bay	1.83±0.03[b]
A3	Asembo Bay	1.79±0.03[b]
Control	-	0.00±0.00[c]
p value		< 0.0001
LSD ($p\leq0.05$)		1.83
CV (%)		15.9671

NOTE: Means followed by the same letter are not significantly different ($p\leq0.05$)

In terms of antibacterial performance of *Actinomycetes* isolates from this study against individual test bacteria, some level of inhibition was observed, which differed significantly ($p<0.05$) for each isolate. Isolate MT3 showed comparatively higher antibacterial activity against 4 out of the 5 bacterial pathogens tested (Figure 3). Isolate MT3 had the highest inhibition against *Staphylococcus* sp. at 12.9 mm followed by *Streptococcus* sp. (9.9 mm), however it did not inhibit the growth of *E. coli* species. The second highest inhibition (10.9 mm) was observed on isolate K3 against *Staphylococcus* sp. Of all the test bacteria, *E. coli* was the most resistant pathogen, inhibited only by isolate A3 at 3.0 mm. Seven isolates were active against *Staphylococcus* species, six against *Streptococcus* species, three against *Xanthomonas* species, two against *Shigella* and two against *E. coli*. A strong inhibition was shown towards *Staphylococcus* species while a weak inhibition was shown towards *E. coli*

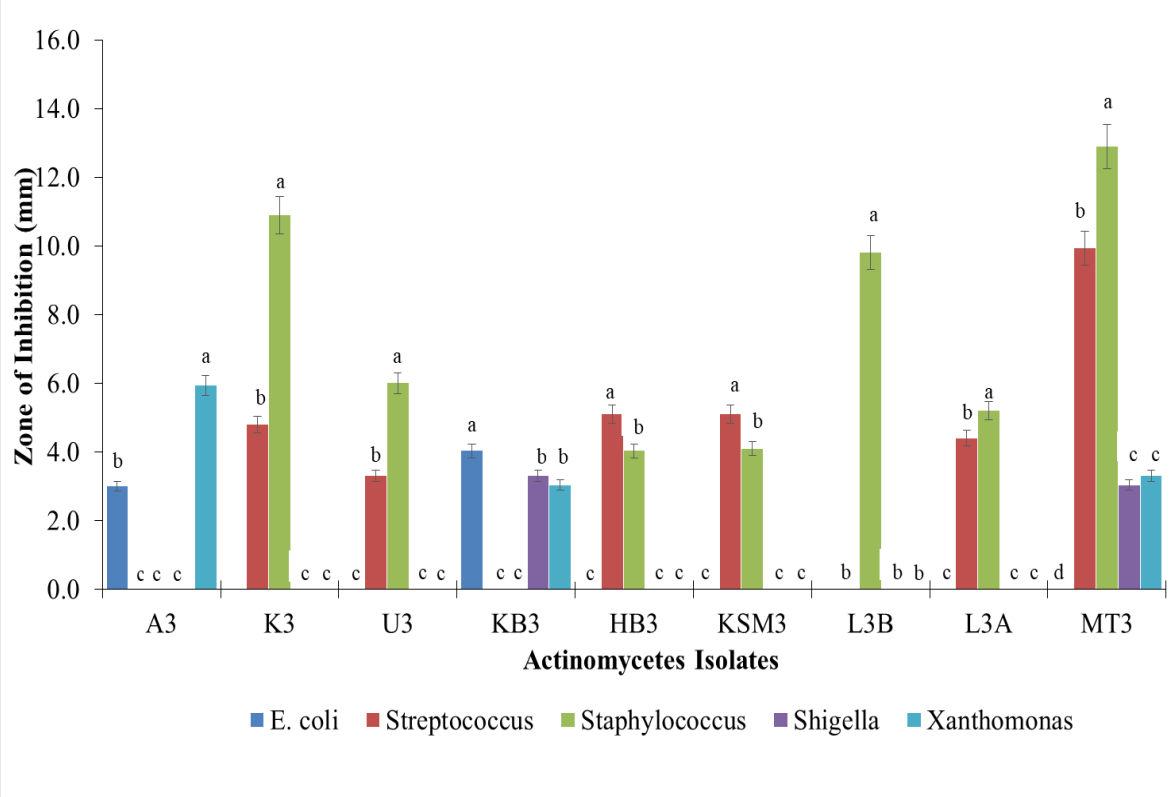

Figure 3. Antibacterial activity (shown by zones of inhibition) of nine *Actinomycetes* isolates from sediments obtained from shores of Winam Gulf, Lake Victoria

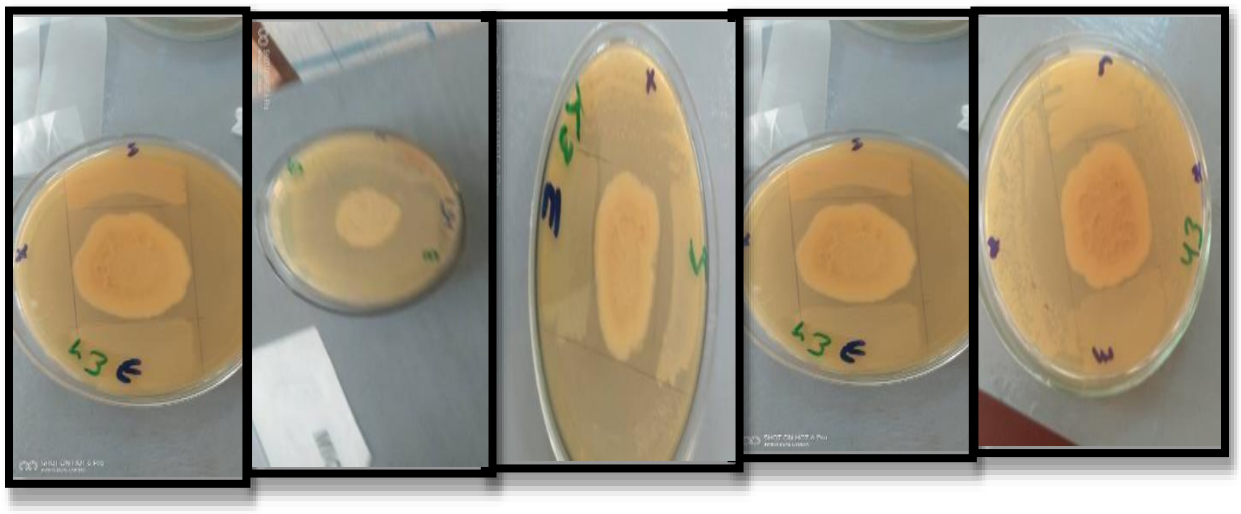

Plate 1: Antimicrobial activity of selected isolated *Actinomycetes* isolates from this study against *Staphylococcus*, *E. coli*, X*anthomonas* species and *Streptococcus* species

4.3 Characterization of Bioactive *Actinomycetes*

4.3.1 Morphological and Biochemical Characterization

A total of 16 *Actinomycetes* were isolated from 24 sediment samples based on their colony morphology. The colonies exhibited different aerial mycelia colors ranging from brown, white, yellow, and grey, while the mycelia within the substrates had brown, black, yellow, orange, and grey colors, as shown by the representative isolates (Table 4.5 and Plate 1). Majority of the colonies had textures that were hard and difficult to scrape off the agar, which are characteristic features of *Actinomycetes* (Salam and Rana, 2014). Microscopically, the colonies were observed to be fragmented hyphae, differentiated branched or filamentous hyphae, and all were Gram and catalase positive. Although 16 isolates were obtained, only 9 isolates were included in the table. This is because only 9 isolates exhibited bioactive properties and were further characterized for their morphological and biochemical traits. These bioactive isolates were prioritized due to their potential to produce bioactive compounds, making them of particular interest for further biochemical and molecular characterization.

Table 4.5 Morphological characteristics of *Actinomycetes* from different sites in the shores of Lake Victoria

Isolate	Site of isolation	Color of aerial mycelium	Color of substrate mycelium	Microscopic hyphal form	Catalase	Gram stain
A3	Asembo Bay	Yellow	Brown	Fragmented hyphae	+	+
U3	Usenge Beach	Black	Black	Differentiated branched hyphae	+	+
MT3	Mbita Beach	White	Yellow	Filamentous hyphae	+	+
K3	Kunya Beach	White	White	Differentiated branched hyphae	+	+
KB3	Kendu Bay	Orange	yellow	Filamentous hyphae	+	+
KSM3	Kisumu Bay	Grey	Grey	Fragmented hyphae	+	+
L3B	Luanda Kotieno Beach	Yellow	Grey	Differentiated branched hyphae	+	+
L3A		Orange	White	Differentiated branched hyphae	+	+
HB3	Homa Bay	Black	Yellow	Filamentous hyphae	+	+

Key: A3-Asembo Bay, HB3- Homa Bay, U3-Usenge Beach, MT3- Mbita Beach, K3-Kunya Beach, KB3- Kendu Bay, KSM-Kisumu Bay, L3A-Luanda Kotieno

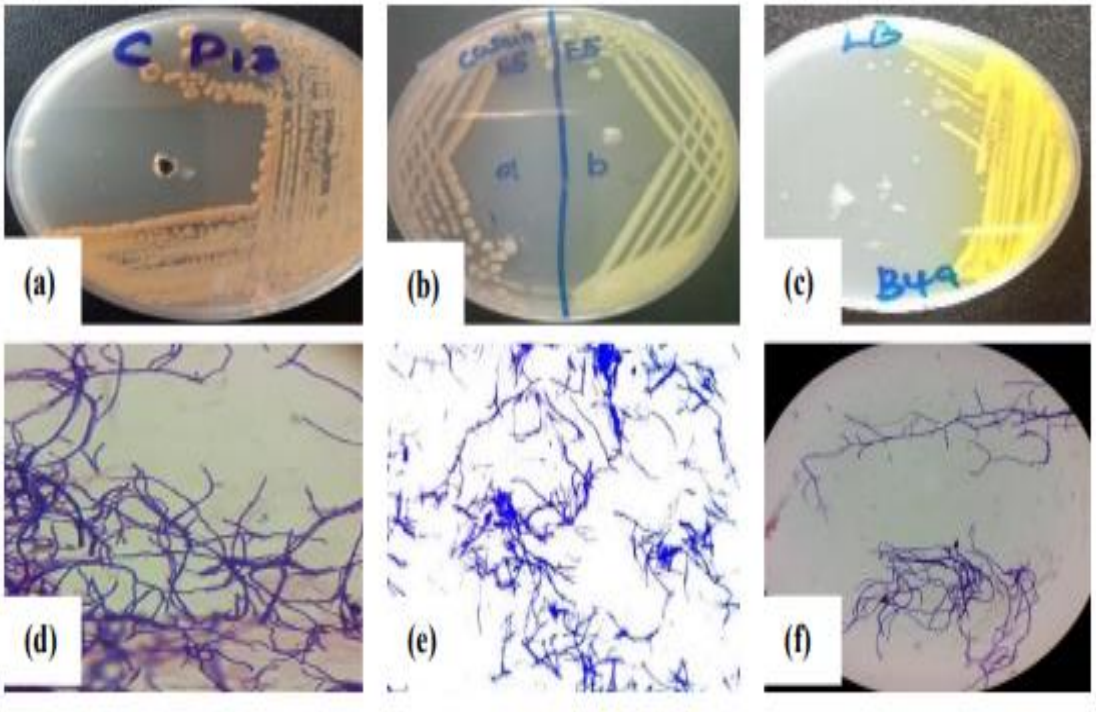

Plate 2. Microscopic and macroscopic characters of pure *Actinomycetes* isolates cultivated on YEMA. NOTE: (a) Orange colony of KB3; (b) Orange colony of L3A and yellow colony of A3; (c) Yellow colony of L3B; (d) Filamentous hyphae of MT3; (e) Fragmented hyphae of KSM3; (f) Differentiated branched hyphae of K3.

4.3.2 Molecular Characterization of Bioactive Actinomycetes Isolates

The 16S rRNA genes of *Actinomycetes* obtained from sediments of Lake Victoria had a molecular weight of about 500bp along a 1kb ladder (Plate 3). On similarity search using the BLAST analys tool, all the isolates had above 99% identity match to those already deposited in the NCBI Gene Bank database, with members of the genus *Streptomyces* being dominant (Table 4.6). However, the isolates were closely related to four different species identified as *Streptomyces microflavus*, *Streptomyces celluloflavus*, *Streptomyces werraensis*, and *Streptomyces cellulosae*.

Nine isolates were sequenced, but only five sequences were successfully processed and submitted to the National Centre for Biotechnology Information (NCBI) Gene Bank, receiving accession numbers ranging from OK560092.1 to OK560096.1. This discrepancy in the number of sequenced isolates and the results received could be due to issues such as sequencing errors, poor-quality DNA, or other technical challenges during the sequencing process.

Plate 3. Gel-images of 16S rRNA genes of six *Actinomycetes* isolates obtained from Lake Victoria shores

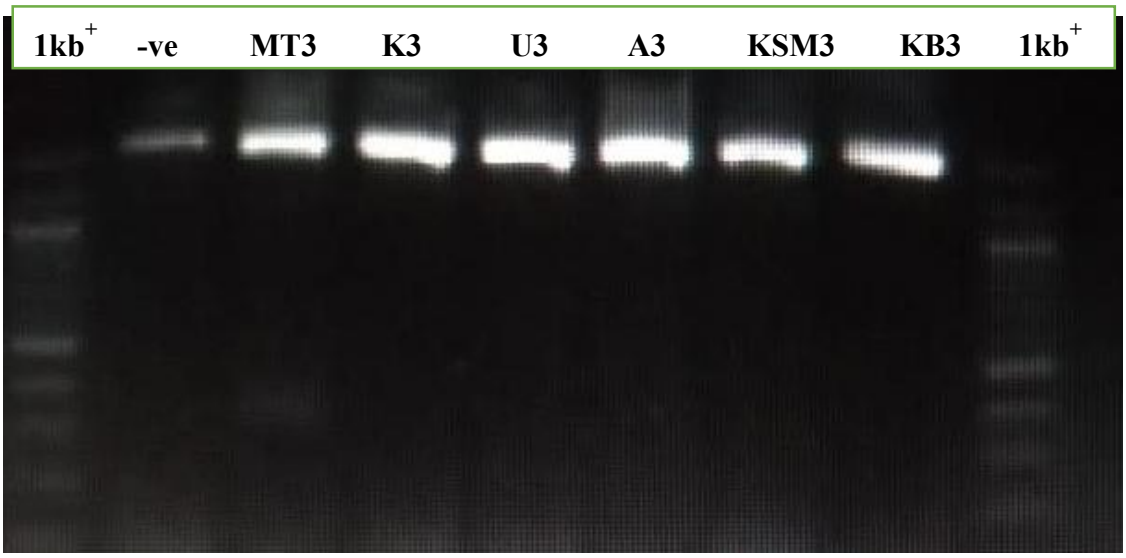

Table 4.6 Identities of 5 *Actinomycetes* isolates obtained from sediments from Lake Victoria shores, based on 16SrRNA gene sequences and similar sequences obtained from NCBI

Isolate Identity	NCBI Acc. No. (this study)	Site of isolation	Similarity %	Species Identity	Similar NCBI Acc. No.	Country of origin	Source of origin
KB3	OK560095.1	Kendu bay	100	*Streptomyces spp.*	MK757965.1	China	Lake sediments
MT3	OK560092.1	Mbita	100	*Streptomyces spp.*	MT260401.1	China	Lake soil
U3	OK560094.1	Usenge beach	99.71	*Streptomyces spp.*	JQ066794.1	China	Lake soil
K3	OK560093.1	Kunya beach	99.93	*Streptomyces spp.*	KX279567.1	Algeria	Lake water
KSM3	OK560096.1	Kisumu bay	100	*Streptomyces spp.*	LC514431.1	Indonesia	River banks

4.3.3 Phylogenetic Analysis of the 16SrRNA sequences

The evolutionary profile conducted using Molecular Evolutionary Genetic Analysis (MEGA 6.0) software clustered the isolates into three clades (Figure 4). Clade I divided into two sub -clusters,

the first sub cluster comprised of isolates MT3 (Acc No.OK560092.1) and KB3 (Acc No. OK560095.1) from sediments obtained from Mbita and Kendu bay respectively and were identified as *Streptomyces microflavus.*They clustered with *Streptomyces sp.* (Acc.No.MT260401.1) and *Streptomyces* sp. (Acc No. MN907704.1) from the gene bank at 88% bootstrap support. The second sub-cluster consisted of isolate U3 (Acc No. OK560094.1) from Usenge which was identified as *Streptomyces celluloflavus* clustered with *Streptomyces celluloflavus* (Acc No. JQ066794.1) and *Streptomyces sp.* (Acc No. KM220610.1) from the gene bank at 88% bootstrap support. Clade II comprised of isolate KSM3 from Kisumu Bay (Acc. No. OK560096.1) which was identified as *Streptomyces cellulosae* clustered with *Streptomyces cellulosae* (Acc No. LC514431) and *Streptomyces gancidicus* (MH819728.1) from the gene bank at 100% bootstrap support. Clade III comprised of Isolate K3 from Kunya Beach (Acc No. OK560093.1) which was identified as *Streptomyces waraensis* clustered with *Streptomyces waraensis (*Acc No. HQ607432.1) and *Streptomyces* (Acc No. KX279567.1) from the gene bank at 84% bootstrap support.

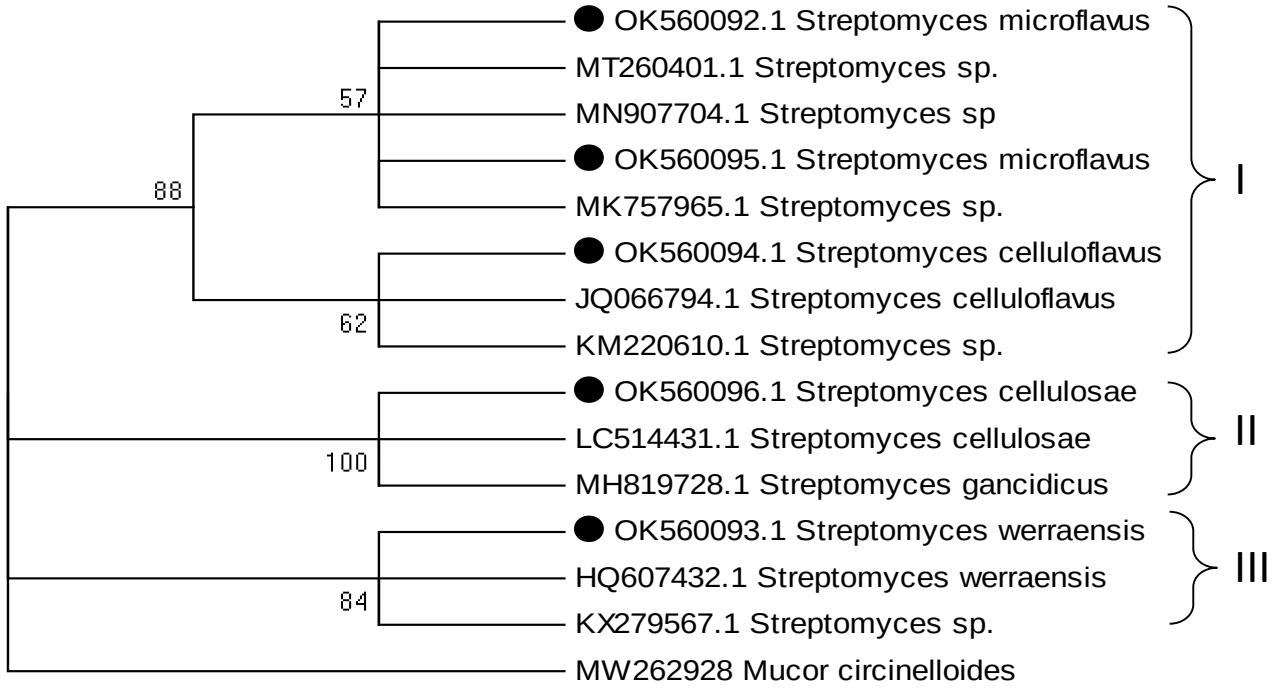

Figure 4. A Neighbor-Joining phylogenetic tree showing *Actinomycetes* isolates from sediments of Lake Victoria basin, compared with sequences retrieved from the NCBI database, with *Mucor circinelloides* as an outgroup root. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Clade I-III shows how the sequences of the *Actinomycetes* isolate clustered based on their similarity.

CHAPTER FIVE
DISCUSSION

5.1 Evaluation of *Actinomycetes* Growth Media

In this study 24 sediment samples from Winam Gulf of Lake Victoria shores successfully generated 16 *Actinomycete* cultures on four different media (Yeast-Malt Extract Agar, Soya Casein Agar, Peptone B Agar and Yeast Extract Agar). A comparison of the media after 10 days of incubation showed that Yeast Malt Extract Agar supported the highest growth at 14.31 mm, compared to Yeast Extract Agar which had the least growth rate of 10.39 mm. The faster mycelia growth observed in Yeast-Malt Extract Agar may be attributed to its superior nutritional content. The manufacturer (Thermofischer Inc® USA) indicates that it contains dextrose (10.0 g), peptone (5.0 g), malt extract (3.0 g), yeast extract (3.0 g) adjusted at a pH of 6.2±0.2. Yeast-Malt Extract Agar contains a rich mixture of Malt extract and Yeast extract, providing a combination of carbohydrates, vitamins, amino acids, and growth factors. Malt extract offers a source of maltose and other sugars, which many *Actinomycetes* can readily utilize as carbon sources (Momin, 2020). Additionally, Yeast Extract complements these nutrients by providing essential growth factors and amino acids. This combination creates an optimal nutrient environment for the growth of a wide range of *Actinomycetes*, leading to the highest observed growth on this media (Cappuccino and Welsh, 2018). The pH and other physicochemical properties of Malt Yeast Extract Agar might be more optimal for the growth of many *Actinomycetes*. These organisms often prefer slightly alkaline conditions, and any deviation from their preferred environment can hinder growth. Malt Yeast Extract Agar may provide a more suitable pH and overall environment, contributing to the higher growth observed (Cappuccino and Welsh, 2018). These nutrient and pH conditions provided the most appropriate carbon, nitrogen and energy source for the growth *Actninomycetes*, as was previously established (Bawazir *et al*., 2018). In contrast, the reduced growth on Yeast Extract Agar can be attributed to its lack of peptone. Peptone is a crucial nutrient that provides a readily available source of nitrogen and carbon, essential for the growth and development of *Actinomycetes.* The absence of peptone in Yeast Extract Agar have resulted in the observed lower growth rate, underscoring its critical role in promoting microbial growth (El-Karkouri *et al.*, 2019; Tiwari and Shreshtha, 2012). Furthermore, Yeast extract might not provide carbon sources that some *Actinomycetes* needs for robust growth. *Actinomycetes* often thrive on complex organic materials such as those found in Starch Casein Agar (starch) and Soya Casein Agar (soybean meal and casein) (Cappuccino and Welsh, 2018). Additionally, Yeast Extract media might favor the

growth of fastidious organisms or those with specific requirements for growth factors and vitamins. However, the majority of *Actinomycetes* isolated from environmental samples like sediment might not fall into this category, leading to lower growth rates on Yeast Extract media compared to other media with more generalized nutrient profiles (Madigan *et al*., 2010). The pH and other physicochemical properties of Yeast Extract media might not be optimal for the growth of many *Actinomycetes.* These organisms often prefer slightly alkaline conditions, and any deviation from their preferred environment can hinder growth (Cappuccino and Welsh, 2018). In summary, the study confirms that Yeast Malt Extrct Agar is the most effective medium for cultivating *Actinomycetes* from Lake Victoria sediments, owing to its superior nutritional content. The findings also highlight the critical role of peptone in promoting microbial growth and the variability in growth potential among different *Actinomycete* isolates. Future research could focus on optimizing media compositions further and exploring the specific nutritional requirements of different *Actinomycete* strains to enhance their cultivation and potential applications. Furthermore, individual Actinomycete isolates showed varying growth rates on different media. Isolate A3 from Asembo Bay exhibited the highest mycelial length of 14.67 mm after 10 days of incubation, in contrast to isolate L3A from Luanda Kotieno, which showed a growth length of 10.21 mm. The enhanced growth of isolate A3 suggests its superior adaptability to environmental conditions such as temperature and pH, as well as its efficient nutrient assimilation capabilities (Sapkota *et al*., 2020; Gurung *et al*., 2009). This variability in growth potential among different isolates is consistent with previous research that highlights the influence of nutrient concentration and environmental conditions on microbial growth (Zothanpuia *et al*., 2018). Winam Gulf of Lake Victoria has different physicochemical conditions due to point and non-point pollution resulting in nutrient loading, eutrophication and acidification (Ongulu, 2015). Although this study did not undertake the physicochemical analysis of the sediment samples, it would be interesting to evaluate its effect on the distribution of *Actinomycetes* within the study area.

5.2 Screening *Actinomycetes* for Antimicrobial Activity

The study investigated antimicrobial activity of metabolites from 16 *Actinomycetes* isolates against a spectrum of bacterial pathogens, including *Staphylococcus sp., Streptococcus sp., Escherichia coli, Shigella sp., and Xanthomonas sp.* provided by the Kenya Medical Research Institute. The findings revealed that all tested *Actinomycete* isolates exhibited higher growth inhibition activity against the Gram-positive bacteria, *Staphylococcus sp. and Streptococcus sp.* compared to the Gram-negative species including *E. coli, Shigella sp., and Xanthomonas sp.* Notably, the antimicrobial activity against the latter three Gram-negative bacteria did not show

significant differences among them. The enhanced susceptibility of Gram-positive bacteria to *Actinomycetes* metabolites can be attributed to the structural differences between Gram-positive and Gram-negative bacteria. Gram-positive bacteria have a simpler cell wall structure consisting of a thick peptidoglycan layer, which is more easily penetrated by antimicrobial agents. In contrast, Gram negative organisms have an outer lipopolysaccharide constituent in addition to an outer peptidoglycan that makes the cell wall impervious to lipophilic solutes compared to gram positive organisms which only have an outer peptidoglycan layer which is not an effective permeable barrier (Kapur *et al*., 2018). This inherent structural resistance is a key factor in the differential antimicrobial activity observed in this study. Several studies have corroborated these findings, emphasizing the general trend of higher efficacy of antimicrobial agents against Gram-positive bacteria compared to Gram-negative bacteria. For instance, Berdy (2012) noted that *Actinomycetes,* known for producing a wide range of bioactive metabolites, tend to be more effective against Gram-positive pathogens due to their ability to disrupt the peptidoglycan synthesis. A study done by Kapur *et al*., (2018), established that gram positive bacteria were highly inhibited by *Actinomycetes* isolates compared to gram negative bacteria. Furthermore, the metabolites produced by *Actinomycetes* often target specific components of the bacterial cell wall, leading to higher efficacy against Gram-positive species (De Simeis and Serra, 2021). On the other hand, lower inhibition towards gram negative could also be attributed to the fact that gram negative bacteria have higher levels of transport proteins, which removes toxic substances such as antibiotics which are produced by *Actinomycetes* (Kumar and Schweizer, 2005). Studies have also established that certain gram negative bacteria acquire antibiotic resistance through mutation or acquisition of foreign DNA through, gene transfer (Jubeh *et al.,* 2020). Lack of significant differences in antimicrobial activity among *E. coli, Shigella sp*., and *Xanthomonas sp.* suggests a uniform level of resistance across these Gram-negative bacteria to the metabolites produced by the *Actinomycete* isolates. This observation aligns with the findings of Cross *et al.*, (2019), who reported that the outer membrane of Gram-negative bacteria provides a robust defense mechanism, often necessitating higher concentrations or more potent combinations of antimicrobial agents to achieve effective inhibition

The differential inhibition observed in this study underscores the importance of targeting Gram-positive pathogens with *Actinomycete*-derived metabolites. However, it also highlights the need for continued exploration of novel strategies to overcome the intrinsic resistance mechanisms of Gram-negative bacteria. Future research could focus on optimizing the production and modification of *Actinomycete* metabolites to enhance their efficacy against resistant Gram-

negative pathogens. In conclusion, the study confirms the higher susceptibility of Gram-positive bacteria to *Actinomycete* metabolites, attributed to the structural differences in bacterial cell walls. While Gram-negative bacteria exhibited uniform resistance, the findings underscore the potential of *Actinomycetes* metabolites as effective agents against Gram-positive infections and the need for further research to improve their activity against Gram-negative bacteria. This insight into the differential antimicrobial activity of *Actinomycetes* metabolites could inform the development of targeted therapeutic strategies, contributing to more effective management of bacterial infections.

5.3 Characterization of Bioactive *Actinomycetes*

5.3.1 Morphological Characterization

The main taxonomic characters used to distinguish between *Actinomycetes* was the cultural, microscopic morphology, biochemical and molecular properties. In terms of microscopic morphology majority of the isolates from this study had permanent and highly differentiated hyphae, typical of *Streptomyces* species. The colonies had different coloration, this may be linked to the difference in utilization of the components within the media. Based on the report given by Sinha *et al.* (2017), colony colors of different microorganisms vary because of synthesising pigments as secondary metabolites while metabolising different nutritional components of the media. As microorganisms grows in culture media they synthesise pigments to protect the cells from dangerous effect of light rays of visible and near ultraviolet range which could be the reason of different pigmentation amongst the *Actinomycetes* isolates. Coloration of the colonies could also provide important references in the determination of new species. The colonies were difficult to scrap off from the media, this may imply that the mycelia were deeply penetrated into the media hence became difficult to scrap off.

5.3.2 Biochemical Characterization

The catalase test performed in this study revealed that all the *Actinomycete* isolates were catalase positive. The presence of catalase enzyme in these isolates is a significant finding, as it provides insight into their metabolic capabilities and potential environmental adaptations (Garcia *et al*., 2021).

Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). This reaction is crucial for protecting cells from oxidative damage caused by reactive oxygen species (ROS), such as hydrogen peroxide, which can be generated during normal metabolic processes or under stressful conditions (Bratovcic *et al.*,2020). The positive catalase reaction observed in all isolates indicates that these *Actinomycetes* possess an effective

mechanism to detoxify hydrogen peroxide, ensuring their survival in environments where oxidative stress might be prevalent.

The presence of catalase is a common characteristic among aerobic and facultatively anaerobic microorganisms, including many species of *Actinomycetes*. These bacteria are known for their ability to thrive in diverse and often challenging environments, such as soil, where they play a vital role in organic matter decomposition and nutrient cycling (Shanthi, 2021). The catalase activity in these isolates likely contributes to their ecological success by enabling them to cope with the oxidative stress associated with their natural habitats.

Catalase positivity can also be linked to the metabolic versatility of *Actinomycetes.* These bacteria are known for their complex and diverse metabolic pathways, which allow them to produce a wide range of secondary metabolites, including antibiotics, enzymes, and other bioactive compounds (Bérdy, 2005). The ability to efficiently manage oxidative stress through catalase activity supports their extensive metabolic functions and enhances their ability to produce these valuable compounds. This is particularly relevant for *Streptomyces* species, which are renowned for their prolific production of antibiotics and other therapeutic agents (Hopwood, 2007). Furthermore, catalase test serves as a useful diagnostic tool in microbial taxonomy and identification. Catalase reaction is one of the biochemical tests used to differentiate between different genera and species of bacteria (Reiner, 2010). In the context of this study, the catalase-positive result supports the identification of the isolates as members of the *Actinomycetes* group, particularly the genus *Streptomyces*, which is typically catalase positive (Gopalakrishnan *et al*., 2020).

The consistent catalase positivity among the isolates also suggests a level of uniformity in their physiological traits, despite their genetic diversity as revealed by 16S rRNA gene sequencing. This finding highlights the importance of combining molecular and biochemical methods for a comprehensive understanding of microbial characteristics and their potential applications. In summary, the positive catalase test results for all *Actinomycete* isolates indicate their ability to detoxify hydrogen peroxide, reflecting their metabolic adaptability and ecological resilience. This enzymatic activity supports their survival in oxidative environments and enhances their capacity to produce valuable secondary metabolites. The catalase test also aids in the taxonomic identification of these isolates, reinforcing their classification within the Actinomycetes group.

The Gram test conducted on the *Actinomycetes* isolates revealed that all of them were Gram-positive. This finding is consistent with the known characteristics of *Actinomycetes*, particularly those within the genus *Streptomyces,* which are typically Gram-positive bacteria.

Gram-positive bacteria are distinguished by their thick peptidoglycan layer in the cell wall, which retains the crystal violet stain used in the Gram staining procedure (Rohde, 2019). This structural feature imparts a purple color to the cells when viewed under a microscope. The thick peptidoglycan layer not only provides structural integrity and protection against environmental stress but also plays a critical role in the bacteria's ability to interact with their environment (Wang *et al*., 2022). The consistent Gram-positive result across all isolates supports their classification within the *Actinomycetes*, specifically the genus *Streptomyces*. *Streptomyces* species are known for their complex multicellular structures and ability to form branching filaments or mycelium, which can be observed under the microscope during the Gram staining process (Hungund *et a*l., 2022). These morphological traits, combined with the Gram-positive cell wall structure, are hallmark features of *Streptomyces* and related genera.

The Gram-positive nature of these isolates also has implications for their ecological roles and biotechnological applications. Gram-positive bacteria, particularly *Actinomycetes*, are renowned for their ability to produce a wide range of secondary metabolites, including antibiotics, antifungals, and enzymes (Bérdy, 2005). The thick peptidoglycan layer and the associated teichoic acids in Gram-positive bacteria can influence the synthesis and secretion of these bioactive compounds, contributing to the ecological success and industrial relevance of these microorganisms (Jubeh *et al*., 2020). In ecological terms, the Gram-positive cell wall structure enhances the ability of *Actinomycetes* to survive in diverse and often challenging environments, such as soil and sediments. The robust cell wall provides resistance to desiccation, high salt concentrations, and other environmental stressors, allowing these bacteria to play a crucial role in organic matter decomposition and nutrient cycling (Sharma *et al*., 2014). Their presence in sediment samples from Lake Victoria highlights their adaptability and ecological significance in such habitats.

Moreover, the Gram-positive nature of these isolates may also affect their interactions with other microorganisms and higher organisms in their environment. Gram-positive *Actinomycetes* are known to produce antimicrobial compounds that can inhibit the growth of competing microorganisms, thereby shaping microbial communities and contributing to soil health and plant

growth (Shanthi, 2021). Understanding the Gram status of these isolates helps in predicting their ecological interactions and potential applications in agriculture and medicine.

In summary, the Gram-positive result for all *Actinomycete* isolates confirms their classification within the *Actinomycetes,* particularly the genus Streptomyces. This structural characteristic is integral to their ecological adaptability, metabolic capabilities, and biotechnological potential. The Gram-positive cell wall provides these bacteria with the resilience needed to thrive in diverse environments and supports their ability to produce valuable secondary metabolites.

5.3.3 Molecular Characterization

Molecular characterization of *Actinomycetes* isolates from Lake Victoria sediments using 16S rRNA gene revealed high sequence identity with known *Actinomycetes* species in the GenBank database. Specifically, isolates KB3, MT3, U3, K3, and KSM3 exhibited over 99% match identity with the following species: S*treptomyces microflavus, Streptomyces microflavus, Streptomyces celluloflavus, Streptomyces werraensis,* and *Streptomyces cellulosae*, respectively. Based on these results, the isolates were assigned the corresponding species names and accession numbers: *S. microflavus* (OK560095.1), *S. microflavus* (OK560092.1), *S. celluloflavus* (OK560094.1), *S. werraensis* (OK560093.1), and *S. cellulosae* (OK560096.1).

The high sequence identity (>99%) observed in the 16S rRNA gene sequences of these isolates indicates a close genetic relationship with the referenced *Streptomyces* species. The 16S rRNA gene is a highly conserved region within the bacterial genome, making it a reliable marker for identifying and classifying bacterial species (Clarridge, 2004). The near-perfect match with known species in the GenBank database confirms the identity of these isolates and provides strong evidence for their taxonomic classification. High similarity match with gene bank isolates could be attributed to the similarities in their gene sequence. This is because species which are closely related do share a common evolutionary lineage. Molecular characterization of microorganisms using 16S rRNA and ITS rDNA gene nucleotide sequences provides organisms with specific signature and hence is considered as very effective method of identifying microbes (Bind and Nema, 2019)

The identification of these isolates as different *Streptomyces* species is significant, given the genus *Streptomyces* is renowned for its ability to produce a wide variety of secondary metabolites, including antibiotics, antifungals, and antitumor agents (Jeya *et al*., 2013; Muthu *et al*., 2013). *Streptomyces microflavus,* for example, has been reported to produce several bioactive compounds

with potential applications in medicine and agriculture (Wang *et a*l.,2011). Similarly, *Streptomyces celluloflavus and Streptomyces cellulosae* are known for their cellulolytic activities, which could be valuable in biomass degradation and biofuel production(Chater,2016). *Streptomyces werraensis* has also been identified as a producer of antimicrobial compounds (Mohamed., 2021). Assigning accession numbers to these isolates (OK560095.1, OK560092.1, OK560094.1, OK560093.1, and OK560096.1) not only validates their identification but also makes them accessible for future research and reference. This contributes to the global repository of genetic information, facilitating further studies on their genetic, biochemical, and ecological characteristics. The availability of these sequences in public databases enhances the reproducibility of research and allows other scientists to compare their findings with these characterized isolates.

The successful identification and classification of these *Actinomycete* isolates underscore the importance of molecular tools in microbial taxonomy. Traditional morphological and biochemical methods, while valuable, can be limited by the phenotypic plasticity of microorganisms and the influence of environmental conditions on their expression. Molecular methods, such as 16S rRNA gene sequencing, provide a more accurate and reliable means of identifying and classifying microorganisms (Bind and Nema, 2019).

In conclusion, the molecular characterization of Actinomycete isolates from Winam Gulf of Lake Victoria sediments has revealed their close genetic relationship with known *Streptomyces* species. This identification, supported by high sequence identity and validated by accession numbers, highlights the potential of these isolates for producing valuable bioactive compounds. The integration of molecular tools in microbial taxonomy is essential for accurate identification and classification, thereby advancing our understanding of microbial diversity and its applications (Rastogi and Sani, 2011).

5.4 Phylogenetic Analysis

Clustering of *Streptomyces microflavus*, *Streptomyces celluloflavus*, *Streptomyces werraensis*, and *Streptomyces cellulosae* into distinct clades with high bootstrap values above 80% provides robust evidence of their phylogenetic relationships. High bootstrap support underscores the reliability of the phylogenetic tree, suggesting that the genetic markers used are effective in distinguishing these species (Tamura *et al*., 2013). Organisms clustering in the same clade could be having similar sequences with vital functional or structural likeliness, therefore being close to each other in a phylogenetic tree. Resemblance in the sequences indicates lack or low levels of mutation that could

lead to reorganization in the nucleotide sequences resulting into divergence in the sequences (Munjal *et al.,* 2018). The method is highly recommended for quick and accurate identification of both culture dependent and culture independent microorganisms (Ikeda *et al*., 2013). This differentiation corroborates their taxonomic status as distinct species, aligning with previous studies that emphasize the importance of molecular data in *Streptomyces* classification (Labeda *et al*.,2012). Ecologically, the distinct clades reflect the diverse metabolic capabilities and ecological roles of these species, such as antibiotic production, organic matter decomposition, and plant growth promotion (Goodfellow & Williams, 1983). This separation suggests that these species may occupy different ecological niches or possess unique functional traits, as seen with the cellulolytic activity of *S. cellulosae(*Regina,2014). From a biotechnological perspective, the genetic and metabolic distinctiveness of these clades, supported by high bootstrap values, highlights their potential for developing novel antibiotics, enzymes, and other bioactive compounds (Baltz,2007). Future research should aim to further characterize the genetic and phenotypic traits of these clades to uncover specific genes and metabolic pathways responsible for their diversity, as well as explore the environmental conditions driving their diversification (Willis and Woodhouse,2020) .In summary, the high bootstrap support for the clustering of these *Streptomyces* species into distinct clades confirms the robustness of the phylogenetic analysis, highlighting their genetic distinctiveness and potential applications in biotechnology. This clustering underscores the phylogenetic, taxonomic, and ecological significance of these species, suggesting avenues for future research and industrial exploitation.

5.5 Limitations of the study

- **Evaluation of in-vitro growth on different media**

Limited representativeness: The study might not encompass the full diversity of *Actinomycetes* present in Winam Gulf of Lake Victoria sediments due to constraints in sampling or culturing methods.

Lack of environmental factors: The in-vitro conditions may not fully mimic the complex environmental factors influencing *Actinomycetes* growth in their natural habitat.

Resource constraints: Restricted access to specialized media or equipment could limit the variety of media tested, potentially overlooking optimal growth conditions for certain strains.

- **Screening for antibacterial activity against selected pathogens**

Narrow spectrum of pathogens: The selected pathogenic bacteria may not fully represent the range of microbial threats encountered in real-world environments, leading to a limited understanding of the *Actinomycetes*' potential applications.

Single screening assay: Reliance on a single antibacterial assay might overlook certain mechanisms of antimicrobial action or fail to detect activity against specific bacterial strains.

- **Characterization of active strains using morphological, biochemical, and molecular markers**

Incomplete characterization: The selected markers may not provide a comprehensive understanding of the biological diversity and ecological roles of the *Actinomycetes* strains.

Methodological limitations: Certain morphological, biochemical, or molecular techniques used for characterization may have inherent biases or limitations, potentially leading to incomplete or inaccurate assessments of strain properties.

CHAPTER SIX
CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusions

- The growth of *Actinomycetes* isolated from sediments obtained from shores of Lake Victoria was dependent on the type of culture media used. Yeast-Malt Extract Agar and Soya Casein Agar media influenced the highest growth of all *Actinomycetes.*
- The efficacy of metabolites from 16 *Actinomycetes* isolates was tested against *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Shigella* spp. and *Xanthomonas* spp. It was observed that nine *Actinomycetes* isolates had better inhibition of the growth of *Staphylococcus sp*. and *Streptococcus sp.* which are Gram positive compared to *E. coli, Xanthomonas species* and *Shigella species* which are Gram negative. Overall, isolate MT3 from Mbita and K3 from Kunya beach were the most efficacious against all the test pathogens.
- Characterization results revealed diverse morphological, biochemical, and molecular profiles among the nine bioactive *Actinomycetes* isolates. However, sequencing yielded results for only five isolates, all identified as belonging to the genus *Streptomyces* and distributed across four species: *Streptomyces microflavus, Streptomyces celluloflavus, Streptomyces werraensis, and Streptomyces cellulosae.* These findings highlight the genetic and phenotypic variability within the *Actinomycetes* community sourced from Winam Gulf of Lake Victoria sediments.

6.2 Recommendations

- Yeast-Malt Extract Agar and Soya Casein Agar media are recommended for growth culture *Actinomycetes* from the study area.
- *Actinomycetes* isolates from this study were bioactive against all the five tested bacteria. Isolates MT3 and K3 were the most efficacious and are recommended for further studies
- Molecular characterization studies using the 16S rRNA gene revealed different species of the genus *Streptomyces.* Morphological and biochemical features could not adequately distinguish between some isolates; which makes molecular markers indispensable in such diversity studies.

6.3 Suggestions for Further Research

- Further investigations on environmental factors such as incubation temperature and moisture requirements for *Actinomycetes* growth is essential for mass cultivation of the bioactive isolates.
- The bioactive compounds from the elite isolates MT3 and K3 should be chemically characterized for possible use in the manufacture of novel antibiotic drugs.
- Further molecular work is necessary using alternative genes to characterize *Actinomycetes* isolates from Lake Victoria basin

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APPENDICES

Appendix I: Comparison of growth rate (mm) on different media

	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	38	1627.425926	42.826998	101.50	<.0001
Error	177	74.680556	0.421924		
Corrected Total	215	1702.106481			

R-Square	Coeff Var	Root MSE	GROWTH Mean
0.956125	5.361264	0.649557	12.11574

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Isolates	8	421.0648148	52.6331019	124.75	<.0001
Replication	2	1.0370370	0.5185185	1.23	0.2951
Media	3	544.0509259	181.3503086	429.82	<.0001
DAYS	1	616.7824074	616.7824074	1461.83	<.0001
Isolates*Media	24	44.4907407	1.8537809	4.39	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Isolates	8	421.0648148	52.6331019	124.75	<.0001
Replication	2	1.0370370	0.5185185	1.23	0.2951
Media	3	544.0509259	181.3503086	429.82	<.0001
DAYS	1	616.7824074	616.7824074	1461.83	<.0001
Isolates*Media	24	44.4907407	1.8537809	4.39	<.0001

Appendix 1I: Constituents of Media and Preparation of Ingredients **grams per litre**

Peptone	10.000 HM
peptone B	3.000
Sodium chloride	5.000
Agar	15.000

Preparations

Suspend 33.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Yeast –Malt extract agar	**grams per litre**
Yeast extract	4.0
Malt extract	10.0
Dextrose	4.0

Agar, 20.0.

Preparations

Suspend 38 grams in 1000 ml distilled water .Heat to boiling to dissolve the medium completely.

Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Soya casein Agar	**grams per litre**
Tryptone	15.000
Soya peptone	5.000
Sodium Chloride	5.000
Agar	15.000

Preparations

Suspend 40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.

Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Nutrient Agar

Lab-Lemco' powder……..1.0g

Yeast extract…….2.0g

Peptone……5.0g

Sodium Chloride…….5.0g

Agar……15.0g

Preparation

28g was suspended in 1 litre of distilled water. This was then heat to boiling point to completely dissolve the medium. Thereafter it was autoclaved at 121°C for 15 minutes allowed to cool then 20ul was aseptically dispensed into sterile Petri plates.

Mueller Hinton Agar (g/L)

Beef, dehydrated infusion from……30.0g

Casein hydrolysate…….17.5g

Starch…….1.5g

Agar……17.0g

Preparation

38g was suspended in 1 litre of distilled water. This was then heat to boiling point to completely dissolve the medium. Thereafter it was autoclaved at 121°C for 15 minutes allowed to cool then 20ul was aseptically dispensed into sterile Petri plates.

Appendix III: ZR Fungal/Bacterial DNA MiniPrep ™ Short Protocol

1. Add 50 – 100 mg (wet weight) fungal or bacterial cells that have been resuspended in up to 200 µl of water or isotonic buffer (e.g., PBS) or up to 200 mg of tissue to a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm). . Add 750 µl Lysis Solution to the tube.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 5 minutes.
3. Centrifuge the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) in a microcentrifuge at 10,000 x g for 1 minute.
4. Transfer up to 400 µl supernatant to a Zymo-Spin™ IV Spin Filter (Orange Top) in a Collection Tube and centrifuge at 7,000 x g for 1 minute.
5. Add 1,200 µl of Genomic Lysis Buffer to the filtrate in the Collection Tube from Step 4.
6. Transfer 800 µl of the mixture from Step 5 to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute.
7. Discard the flow through from the Collection Tube and repeat Step 6.
8. Add 200 µl DNA Pre-Wash Buffer to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute.
9. Add 500 µl g-DNA Wash Buffer to the Zymo-Spin™ IIC Colum n and centrifuge at 10,000 x g for 1 minute.
10. Transfer the Zymo-Spin™ IIC Column to a clean 1.5 ml microc entrifuge tube and add 100 µl (35 µl minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA

Appendix IV: Gel electrophoresis protocol

Preparation of the Gel

1. Weigh out the appropriate mass (0.8%) of agarose into an Erlenmeyer flask.
2. Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 Mm EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).
3. Melt the agarose/buffer mixture over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.
4. Add ethidium bromide (EtBr) to a concentration of 0.5 μg.
5. Allow the agarose to cool either on the bench top or by incubation in a 65 °C water bath. Failure to do so will warp the gel tray
6. Place the gel tray into the casting apparatus and place an appropriate comb into the gel mold to create the wells.
7. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box.

2. Setting up of Gel Apparatus and Separation of DNA Fragments

1. Add loading dye to the DNA samples to be separated. Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol). Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel.
2. Program the power supply to desired voltage (1-5V/cm between electrodes).
3. Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.
4. Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working.
5. Remove the lid slowly and carefully load the DNA sample(s) into the gel. An appropriate DNA size marker should always be loaded along with experimental samples.
6. Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads). Double check that the electrodes are plugged into the correct slots in the power supply.
7. Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

3. Observing Separated DNA fragments

1. When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.
2. Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.
3. Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system

Appendix V: Partial FASTA sequences of 16S rRNA genes of *Actinomycetes* from this study

>OK560092.1 *Streptomyces microflavus*

TGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCT
GGAAACGGGGTCTA
ATACCGGATAACACTCTGTCCCGCATGGGACGGGGTTAAAAGCTCCGGCGGTGAAG
GATGAGCCCGCGGC
CTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCT
GAGAGGGCGACCGG
CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATA
TTGCACAATGGGCG
AAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTC
TTTCAGCAGGGAAG
AAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAG
GGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCAC
GTCGGATGTGAAAG
CCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGG
GAGATCGGAATTCC
TGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGAT
CTCTGGGCCATTAC
TGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGTT
GGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGCATTAAGT
TCCCCGCCTGGGG

AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCAGC
GGAGCATGTGGCTTA
ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATATACCGGAAAGCATCAG
AGATGGTGCCCCCC
TTGTGGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGC
AACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTC
ACAGGAGACTGCC
GGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTT
GGGCTGCACACGT
GCTACAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAATCTCAAAA
AGCCGGTCTCAGTT
CGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAGTAATCGCAGATC
AGCATTGCTGCGG
TGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACA
CC

>OK560093.1 Streptomyces werraensis
GCAGTCGAACGATGAAGCCCTTCAGGGGTGGATTAGTGGCGAACGGGTGAGTAACA
CGTGGGCAATCTGC
CCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAACACCCGCCG
AGGCATCTCGGTGG
GTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGGGGT
GATGGCCTACCAA
GGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACA
CGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGC
CGCGTGAGGGATGA
CGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCT
GCAGAAGAAGCGCC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAA
TTATTGGGCGTAAA
GAGCTCGTAGGCGGCTTGTCGCGTCGGATGTGAAAGCTCGGGGCTTAACCCCGGGTC
TGCATTCGATACG

GGCAGGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCG
CAGATATCAGGAGG
AACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGC
GTGGGGAGCGAACA
GGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGAC
ATTCCACGTCGTCG
GTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAA
ACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAA
CCTTACCAAGGCTT
GACATATACCGGAAACGGCCAGAGATGGTCGCCCCCTTGTGGTCGGTATACAGGTG
GTGCATGGCTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCT
GTGTTGCCAGCAT
GCCCTTCGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGG
TGGGGACGACGTCA
AGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAAAG
AGCTGCGAAGCCG
TGAGGCGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTC
GACCCCATGAAGTC
GGAGTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCG
TCACGTCACGAAAGTCGGTAACACC

>OK560094.1 *Streptomyces celluloflavus*

GCAGTCGAACGATGAAGCCTTTACAGGGGTGGATTAGTGGCGAACGGGTGAGTAAC
ACGTGGGCAATCTG
CCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAATACTCCTGC
CTGCATGGGTGGG
GGTTGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGGG
GTAATGGCCTACCA
AGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGAC
ACGGCCCAGACTCCT

ACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGAC
GCCGCGTGAGGGATG
ACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACC
TGCAGAAGAAGCGC
CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGA
ATTATTGGGCGTAA
AGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGG
TCTGCATTCGATAC
GGGCTAGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGC
GCAGATATCAGGAG
GAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAG
CGTGGGGAGCGAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGA
CATTCCACGTCGTC
GGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAA
AACTCAAAGGAATT
GACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGA
ACCTTACCAAGGCT
TGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTATACAGGTG
GTGCATGGCTGTC
GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTC
TGTGTTGCCAGCA
TGCCCTTCGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAG
GTGGGGACGACGTC
AAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAAT
GAGCTGCGATGTC
GTAAGGCGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACT
CGACCCCATGAAGT
CGGAGTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCC
GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGGTGGCCTC

>OK560095.1 *Streptomyces microflavus*

TGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGAC
AAGCCCTGGAAAC
GGGGTCTAATACCGGATAACACTCTGTCCCGCATGGGACGGGGTTAAAAGCTCCGG
CGGTGAAGGATGAG
CCCGCGGCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTA
GCCGGCCTGAGAGG
GCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT
GGGGAATATTGCAC
AATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTG
TAAACCTCTTTCAG
CAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTG
CCAGCAGCCGCGGTA
ATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGG
CTTGTCACGTCGGA
TGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGT
GGTAGGGGAGATC
GGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGA
AGGCGGATCTCTGG
GCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCT
GGTAGTCCACGCCG
TAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACG
CATTAAGTTCCCC
GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCAC
AAGCAGCGGAGCAT
GTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATATACCGGAA
AGCATCAGAGATGG
TGCCCCCCTTGTGGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGAT
GGGGACTCACAGG
AGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCC
TTATGTCTTGGGCT

GCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGCGGAGCGAA
TCTCAAAAAGCCGG
TCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAGTAAT
CGCAGATCAGCAT
TGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGT
CGGTAACACCCGA
AGCCGG
>OK560096.1 *Streptomyces cellulosae*
GTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGG
ATACTGATCGCCTT
GGGCATCCTTGGTGATCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCA
GCTTGTTGGTGAGG
TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC
TGGGACTGAGACAC
GGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCC
TGATGCAGCGACGC
CGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGA
AAGTGACGGTACCT
GCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCG
AGCGTTGTCCGGAA
TTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGG
CTTAACCCCGGGT
CTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGT
AGCGGTGAAATGCG
CAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGC
TGAGGAGCGAAAGC
GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCAC
TAGGTGTGGGCGAC
ATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGG
CCGCAAGGCTAAA
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CGCAACGCGAAGAA

CCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGT
CGGTGTACAGGTG
GTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGTCC
CGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCA
ACTCGGAGGAAGGT
GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAAT
GGCCGGTACAATG
AGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTG
GGGTCTGCAACTCG
ACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATAC
GTTCCCGGGCCTT

Appendix VI: NACOSTI permit to conduct the study in Lake Victoria basin

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY& INNOVATION

Ref No: 19521

Date of Issue: 19/November/2021

RESEARCH LICENCE

This is to Certify that Ms.. PHOEBE AKOTH OBIERO of JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE AND TECHNOLOGY, has been licenced to conduct research in Lake Victoria Regions on the topic: CHARACTERIZATION AND ANTIBACTERIAL ACTIVITIES OF ACTINOMYCETES ISOLATED FROM SEDIMENTS OF LAKE VICTORIA SHORES IN LAKE VICTORIA REGIONS for the period ending: 29/November/2022

Licence No: NACOSTI/P/192453

19521

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

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Appendix VII: Approval to conduct research by JOOUST Ethics and Review Committee

JARAMOGI OGINGA ODINGA
UNIVERSITY OF SCIENCE AND TECHNOLOGY

DIVISION OF RESEARCH, INNOVATION AND OUTREACH
JOOUST-ETHICS REVIEW OFFICE

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P.O. BOX 210 - 40601
BONDO

OUR REF: JOOUST/DVC-RIO/ERC/E2

11th February, 2020

Phoebe Akoth Obiero
SBPS
JOOUST

Dear Ms. Obiero,

RE: APPROVAL TO CONDUCT RESEARCH TITLED *"CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM SEDIMENTS OF LAKE VICTORIA SHORES"*

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal. Your application approval number is **ERC/17/02/20-20.** The approval period is from 11th February, 2020 – 10th February, 2021.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by JOOUST IERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks of affected safety or welfare of study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOUST IERC.

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science, Technology and Innovation (NACOSTI) https:oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Prof. Francis Anga'wa
Chairman, JOOUST ERC
Copy to: Deputy Vice-Chancellor, RIO Director, BPS Dean, SBPS

Appendix VIII: Authority to conduct field work by JOOUST board of postgraduate studies.

JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE & TECHNOLOGY

BOARD OF POSTGRADUATE STUDIES

Office of the Director

Tel. 057-2501804
Email: bps@jooust.ac.ke

P.O. BOX 210 - 40601
BONDO

Our Ref: S152/4204/2018

Date: 22nd January 2020

TO WHOM IT MAY CONCERN

RE: PHOEBE AKOTH OBIERO – S152/4204/2018

The above person is a bonafide postgraduate student of Jaramogi Oginga Odinga University of Science and Technology in the School of Biological, Physical, Mathematics and Actuarial Sciences pursuing Master of Science in Microbiology. He has been authorized by the University to undertake research on the topic: ***"Characterization and Antibacterial Activity of Actinomycetes Isolated from Sediments of Lake Victoria Shores".***

Any assistance accorded to her shall be appreciated.

Thank you.

Prof. Dennis Ochuodho
DIRECTOR, BOARD OF POSTGRADUATE STUDIES