EVALUATION OF ANTIMICROBIAL ACTIVITY IN *ENTEROBACTER*, *PAENIBACILLUS* AND *BREVIBACILLUS* SPECIES ISOLATED IN SOIL SAMPLES FROM LAKES MAGADI, BOGORIA AND ELEMENTAITA

BY

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DECLARATION

Declaration

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree in any other university.

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DEDICATION

This thesis is dedicated to my late father who would have wished to see me pursue my Masters studies but unfortunely he couldn’t. May he continue resting in peace.

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ACRONYMS AND ABBREVIATIONS

AMPs	Antimicrobial peptides
BLAST	Basic Local Alignment Search Tool
CIP	Ciprofloxacin
CTAB	Cetyltrimethylammonium Bromide
d NTPs	Dinucleotide triphosphates
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPS	Exopolysaccharides
$FADH_2$	Dihydroflavine-adenine dinucleotide
FDA	Food and Drug Administration
GTR	Generalized time reversal
HCN	Hydrogen Cyanide
LPS	Lipopolysaccharides
MCMC	Merkov Chain Monte Carlo
Md	Median
MRSA	Methicillin resistant *Staphylococcus aureus*
MUSCLE	Multiple sequence comparison by log-expectation
NADH	Reduced Nicotinamide adenine dinucleotide
PCR	Polymerase Chain Reaction
SDS	Sodium Dodecyl Sulphate
SIM	Sulphur indole motility
TAE	TrisAcetate EDTA
TSI, B/S	Triple Sugar Iron, Butt/Slant

ABSTRACT

Species of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* are widely distributed throughout the globe and they have been isolated in different habitats i.e. soil, saline and alkaline water bodies, decaying plant materials, clinical materials and from the gut of animals and human microbiota. Most species of these genera are known to produce antimicrobial secondary metabolites with wide application in pharmaceutical, agricultural and food industries. The emergence of pathogenic microorganisms with multiple drugs resistance has raised the need to find more alternatives of potential active biological compounds from natural resources. In this study, various species of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* were isolated in soil samples from Lakes Magadi, Bogoria and Elementaita, characterized and tested for their antimicrobial activity against various pathogenic microorganisms with the aim of finding new antimicrobial compounds. Twenty-four isolates of these genera were isolated and fermented in CY broth with amberlite XAD 16 (adsorbent resin) for 7 days at 30° C. The antimicrobial compounds were extracted using methanol and concentrated in rotary evaporator. Nine isolates presented antimicrobial activity against test pathogenic bacteria (*Salmonella spp*., *E. coli*, *Pseudomonas spp*., *K. pneumonia*, *S. aureus* and *L. monocytogenes*). Isolates *Enterobacter sp*. strain MC dung, *Enterobacter sp*. strain BC dung, *Brevibacillus sp*. Strain BC/5BC, FU78CB, *Enterobacter sp*. strain FU 103BA and *Brevibacillus sp*. strain ELHPS_3H_{13} expressed broad spectrum against the test pathogenic bacteria while *Paenibacillus sp*. Strain FU 75 BC, *Enterobacter sp*. FU 104C and BC5AC showed narrow spectrum against Gram-negative bacteria (*Salmonella spp*., *K. pneumonia*, *Pseudomonas spp*., and *E. coli*). Among the isolates, *Enterobacter sp*. strain BC dung had significantly ($P< 0.05$) higher antibacterial activity against *Pseudomonas spp. (Md*=16mm), *S. aureus* (*Md*= 35mm), *Listeria monocytogenes* (*Md*= 34mm) and *K. pneumoniae* (*Md* =30mm). The morphological, biochemical and molecular characterization revealed that isolates BC dung, MC dung, FU 104 C and FU 103 BA belong to genus *Enterobacter*, BC/5BC and ELHPS_3H_{13} belong to genus *Brevibacillus*, while isolates FU 75 BC, FU 78CB and BC5AC belong to genera *Paenibacillus*, *Providencia* and *Lysinibacillus* respectively. The isolated species of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* possessed antimicrobial compounds which were active against the selected pathogenic bacteria.

CHAPTER ONE
INTRODUCTION

1.1 Soda lakes environments and their microbial diversity

Soda lakes are among the major type of alkaline environment in which the microorganisms that dwell in them are subjected to extreme ecological conditions such as high pH, high salt concentration and turbidity (Schagerl and Burian, 2016). These microorganisms are sometimes referred to as extremophiles (Chettri *et al*., 2021). East Africans' soda lakes are the most studied soda lakes throughout the world (Krienitz, 2016). Soda lakes are described by the presence of high Na_2CO_3 concentrations, low levels of Ca^{2+} and Mg^{2+} (Ma *et al*., 2019) and sodium chloride. Nonetheless, most of these soda lakes possess extreme high primary productivity that is reflected by green, orange, red, or pink color because of massive blooms of microorganisms. They have a dense population of aerobic halophilic, organotrophic, alkalophilic, and alkalitolerant bacteria and archaea (Grant and Jones, 2016). Despite high salt concentrations (20% w/v) photosynthetic primary production play a vital role in these environments hence supporting other microbial community (Kambura *et al*., 2016). The hypersaline lakes support both cyanobacteria and alkalophilic anoxygenic phototrophs of genera *Ectithiorhodospira* and *Halorhodospira* while the less alkaline lakes are mainly cynobacteria (Kevbrin, 2019).

1.2 The Genera *Enterobacter*, *Paenibacillus* and *Brevibacillus*

The Genus *Enterobacter* belong to the family *Entrobacteriaceae* and was first described in 1960 (Davin *et al*., 2019). Bacteria in this genus are Gram-negative bacilli with a cell length of about 2µm, they have flagella and usually facultative anaerobes. Under this genus 22 species have been discovered including, *E. aerogenes*, *E. asburiae*, *E. amnigenus*, *E. cloacae*, *E. carcinogenus*, *E. mori*, *E. oryzae* and *E. hormaechei*. The environmental habitats associated with genus *Enterobacter* include soil, water and plants as phytopathogens

(Vojkovska *et al*., 2016). Others are natural commensals of human and animal gut microbiota. Some bacteria of genus *Enterobacter* are associated with serious human infections (Tajeddin *et al*., 2016; Baran, 2016; Friedman *et al*., 2017; Legeay *et al*., 2018). *Enterobacter* strains are among the group of bacteria that lead in cases of resistant nosocomial infections. They are frequently associated with multidrug resistance phenotype (Annavajhala *et al*., 2019). However, recent studies have proved that members of genus *Enterobacter* produce lipopeptides with wide applications in food and pharmaceutical industries (Meena and Kanwar, 2015). Lipopeptides have been approved for use as antibiotics in the USA since 2003. Cubicin that is used in treatment of serious food and skin infections was the first cyclic lipopeptide to be approved by Food and Drug Administration (FDA) in USA (Chen and Lu, 2020). Various lipopeptides produced by the genus *Enterobacter* with broad spectrum of antibacterial activities include; iturin, fengycin, and kurstakin (Jemil *et al*., 2019).

The *Paenibacillus* are Gram negative or Gram variable, anaerobic or facultative anaerobic, rod-shaped, endospore forming bacteria. *Paenibacillus* strains are found in various ecological habitats e.g. water, soil, vegetative matter, tree roots and in hospital clinical materials (Simonová *et al*., 2015; Priest, 2015; Grady *et al*., 2016). Cellulolytic *Paenibacillus* contain cellulase that aid in the hydrolysis of cellulose. *Paenibacillus* species produce metabolites such as, amino acids, polysaccharides, antibiotics agent, toxins, and pigments (Santos *et al*., 2016; Eswari *et al*., 2019; Abd-El-Khair, 2020). The mechanisms that contribute to their antimicrobial activity include competition of nutrients and the membrane disruption of the targetcell (Cochrane and Vederas, 2016). *Paenibacillus* species have antimicrobial activities against various microorganisms such as filamentous fungi (Han *et al*., 2017; Olishevska *et al*., 2019; Daud *et al*., 2019), aerobic bacteria and anaerobic bacteria like *Clostridium*

botulinium (Chau *et al*., 2020). *Paenibacillus* also produce antifungal inhibitors against many plant and animal pathogens. Due to their ability to degrade chitin, major compound present in plant cell walls, they have gained importance in bio-control (Passera *et al*., 2017; Chávez *et al*., 2020). *Paenibacillus* produce ribosome-synthesized bacteriocins (pediocins and Lantibiotics) and non-ribosome synthesized peptides. Lantibiotics are active against Gram negative and some Gram-positive bacteria (Huang, 2015). The non-ribosome synthesized peptides consist of linear cationic, cyclic cationic and cyclic non- cationic which act by disrupting membrane of target cells.

The genus *Brevibacillus* is found in the family *Paenibacillaceae*. Members of this genus are Gram positive, or Gram variable (Yang and Yousef, 2018), with rod-shaped and round ended cells which occur singly, paired or in chains. The *Brevibacillus* include psychrophilic, thermophilic, acidophilic, halophilic and alkalophilic bacteria. They are either heterotrophs (use various carbon source) or autotrophs. The genus *Brevibacillus* is widely distributed in nature occurring on aquatic environments, dust, rocks, and guts of animals including insects (Ray *et al*., 2020). *Brevibacillus* produce gramicidin, a cyclic decapeptide that exerts activity against fungi, Gram-negative bacteria, viruses and single cells pathogenic eukaryotes (Parmar *et al*., 2020; Kumar *et al*., 2018). Some strains of *Brevibacillus* are potential biological control agents because of their antifungal properties. For example, *Brevibacillus brevis* has antifungal activities against *Fusarium wilt* of pigeon pea (Liu *et al*., 2021) while *Brevibacillus laterosporus* is active against wheat foliar necrotrophic. *B. agri*, *B. brevis*, and *B. formosus* are able to suppress root rot diseases and wilt disease in *Salvia officinalis* through the production of inhibition metabolites like chitinase and siderophore. *Brevibacillus* has also been demonstrated to control brown leaf spot in potato (Ahmed 2017). These bacteria also play a significant role in bio-remediation of heavy metal contaminated

environments. For example, *Brevibacillus* species are used in the remediation of the soils contaminated with Arsenic metal (Hussain *et al*., 2021).

1.3 Problem statement

The uncontrolled use of antibiotics in humans, agriculture and animals has led to the wide spread of antibiotic resistance in the community at large (Geta, 2019; Ma *et al*., 2021). Increase in international trade and travel has also accelerated the transfer and spread of resistant strains of microorganisms turning them into global concern. Infections such as tuberculosis, pneumonia, gonorrhea, and food borne diseases have become difficult to cure as antibiotics become ineffective. About 214,000 infants are estimated to lose lives each year from sepsis resulting from resistant bacteria, that is a representative of 30% of all sepsis deaths in infants (Obiero 2022). The emergence of multiple drugs resistant pathogenic microorganisms has raised the need to look for other alternatives of potential biocontrol agents from natural resources. Microorganisms producing bioagents are better alternatives to handle than secondary metabolites from plants. They are easy to manipulate and the production of the active agents can be increased by increasing the fermentation volumes. The turnaround time from culture to fermentation products is also shorter. Additionally, natural products are more environmental friendly compared to synthetic products.

1.4 Research Questions

1. Will there be bacterial strains of the genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* in the soil sediments collected from Lakes Magadi, Bogoria and Elementaita?
2. Do the crude extracts of isolated strains have biological activity against the selected pathogenic bacteria?

1.5 Objectives

1.5.1 Broad objective

To isolate and characterize *Enterobacter*, *Paenibacillus* and *Brevibacillus* from Lakes Magadi, Bogoria and Elementaita and screen their extracts for antibacterial activities

1.5.2 specific objectives

i. To isolate and characterize *Enterobacter*, *Paenibacillus* and *Brevibacillus* from soil sediments in Lakes Magadi, Bogoria and Elementaita
ii. To extract and screen the fermented crude extracts from *Enterobacter*, *Paenibacillus* and *Brevibacillus* for biological activity against selected pathogenic bacteria.

1.5.3 Hypotheses

The soil sediments from Lakes Magadi, Bogoria and Elementaita contain bacteria of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* that produce antibacterial metabolites

1.6 Justification

Soda lakes ecology are biodiversity-rich habitats for microbes especially halophytes and mesophytes yet very little has been studied on these microorganisms to establish their secondary metabolite production potential. This study aimed at isolation, identification, characterization and documentation of the occurrence of indigenous *Enterobacter*, *Paenibacillus* and *Brevibacillus* in Lakes Magadi, Bogoria and Elementaita. This is a preliminary step in exploiting their potential as natural producer of biological active compounds. The utilization of these genera as potential secondary metabolites producers has not been exhausted due to the fact that researchers tend to concentrate much on their pathogenicity and antimicrobial resistance hence overlooking the study of their useful antimicrobial compounds.

CHAPTER TWO
LITERATURE REVIEW

2.1 Soda lake environments and their microbial diversity

Soda lakes are known to have high salinity and stable alkaline pH compared to other types of saline lakes (Felföldi, 2020). They are mainly found in arid and semi-arid regions where the rate of water evaporation is more than the rate of water inflow. They are characterized by high concentration of (Na_2C0_3. $10H_20$ or $Na_2CO_3.NaHCO_3.2H_2O$), low levels of Ca^{2+} and Mg^{2+} (Boros and Kolpakova, 2018) and presence of high levels of chloride ions in form of sodium chloride resulting to their salinity. Soda lakes such as Bogoria, Magadi, Natron, and Elementaita of the East African Rift Valley are the best representatives of soda lake environments in the world. The development of these lakes in Kenya and Tanzania Rift valley is as a result of geological and topographical factors (Kübler *et al*., 2021). The pH ranges from 8.5 to 12 and remains constant throughout the year regardless of the change in seasons. The salt concentration in Lakes Bogoria, Nakuru, Elementaita, Sonachi and Magadi ranges from 5% to35%. (Deocampo and Renaut, 2016).

Despite having hostile environmental conditions, soda lakes feature diverse microbial communities and this is mainly attributed to the high nutrient levels, high temperatures, more light intensities and the presence of thick blooms of cyanobacteria (Fazi *et al*., 2021). There are around 10^6-10^7 cells/mL of planktonic bacteria in the soda lakes which belong mainly to the phyla Bacteriodetes, Actinobacteria and Proteobacteria (Felföldi, 2020). The microbial community of soda lakes is controlled by archaea and bacteria that contribute to the primary production of cycling of nitrogen, sulphur and carbon through aerobic and anaerobic conditions of the soda lakes (Grant and Jones, 2016). Alkalophilic bacillus are abundant in soda lakes where they are involved in the breakdown of bio-polymers with the help of

extracellular hydrolytic enzymes; cellulases, xylamases, proteinases and other enzymes that degrade complex carbohydrates. *Streptomyces spp.* which is a Gram positive bacteria grow at pH 10 and is involved in the hydrolysis of protein and carbohydrates. The bacteria responsible for recycling of nutrients have been identified, for example, Lake Magadi harbours haloalkaliphilic archaea like *Halorubrum vacuolatum*, *Natrialba magadi*, and *Natronobacterium gregoryi* (Orwa *et al*., 2020). These organotrophs require high concentration of sodium chloride, high pH (8.5 -11) and low magnesium ions concentrations (< 10m M).

Soda lakes in Rift valley are habitats of diverse population of anoxygenic phototrophic bacteria, aerobic cyanobacteria, aerobic organotrophic bacteria and non-phototrophic bacteria (Imhoff, 2017). The anaerobic groups that are mostly found in soda lakes include; hydrogenotrophic sulphate reducers, acetogenic ammonifiers, Sulphur oxidizing bacteria and methane oxidizing bacteria (Trutschel *et al*., 2022). Chemoorganotrophic alkalophilic anaerobes such as *Spirochaeta spp.* have also been isolated in Lake Magadi (Kulkarni *et al*., 2019). These groups of bacteria are able to utilize pentoses, hexose and disaccharides to produce ethanol, acetate and hydrogen.

2.2 The Genus *Enterobacter*

The genus *Enterobacter* belongs to the class Gamma *Proteobacteria* and family *Enterobacteriaceae*. Members of genus *Enterobacter* are facultative anaerobes, Gram-negative bacilli and move by help of flagella (Davin *et al*., 2019). Many taxonomic changes have occurred since it was discovered in 1960. There are 22 species that have been discovered in this genus i.e. *E. amnigenus*, *E. aerogenes*, *E. asburiae*, *E. arachidis*, *E. cloacae*, *E. carcinogenus*, *E. cowanii*, *E. gergoviae*, *E. dissolvans*, *E. hormaechei*, *E. helveticus*, *E. ludwigii*, *E.*

kobei, *E. nimipressuralis*, *E. mori*, *E. turicensis*, *E. pulveris*, *E. oryzae*, *E. pyrinus*, *E. soli*, *E. radicincitans*, and *E. taylorae* (Davin *et al*., 2019). *Enterobacter* inhabit natural environment such as soil, water, dung of herbivores and some as phytopathogens for many plants species. Many species have also been found in the respiratory tract, urine, and blood (Sutton *et al*., 2018).

2.2.1. Biochemical and morphological characterization of genus *Enterobacter*

Enterobacter are positive for the Simmons citrate and voges proskauer tests. They are motile and produce gas from D- glucose, L- rhaminose, D- mannitol, L-arabinose, salicin, D-cellobiose, D-xylose, Maltose and trehalose. They are able to hydrolyze O- nirophenyl –β D-galactosidase, reduce nitrate and oxidize D- glucose (Grimont, 2015). Many *Enterobacter* species are positive for carbon dioxide production from glucose and negative for H_2S production from thiosulphate (Thompson *et al*., 2018). They are negative for oxidase test, while catalase test varies among the *Enterobacteriaceae.*

Different *Enterobacter* species are able to hydrolyze cellulose polymers through the production of various cellulolytic enzymes (Sari *et al*., 2017). Colonies of *Enterobacter species* in Congo-red medium grow luxuriantly, they are about 3-5mm in diameter and pink in color with raised elevation after 72 hours of incubation at 30°C. In nutrient agar the isolates appear mucoid, irregular in shape with convex elevation and entire edges. The colonies form swarm on prolonged incubation (Araujo *et al*., 2019).

2.2.2 *Enterobacter* antimicrobial properties

Lipopeptides, glycolipids, polysaccharides, phospholipids, and neutral lipids belong to a group of surface active compounds that are produced through biological metabolism. These active compounds are referred to as biosurfactants. Lipopeptides contain peptides chains and fatty acid and show substantial antimicrobial activities (Neubauer *et al*., 2020). Depending on

their structural differences lipopeptides have been split into 23 families whereby 21 of these were discovered to be cyclic lipopeptides (Zhou *et al*., 2020). Lipopeptides have great potential applications in the food, pharmaceutical, cosmetic, agricultural industries and in microbial enhanced oil recovery. Many studies of the *Enterobacter* species concentrated on its antimicrobial susceptibility and pathogenicity for humans but in rare cases on production of useful metabolites (Jiang *et al*., 2020). However, the recently discovered strains which have been isolated from environmental samples have been studied for their various beneficial properties such as biocontrol of human and plant diseases and promotion of plant growth (Biniarz *et al*.,2017). Various lipopeptides produced by strains of *Enterobacter* with broad spectrum of antibacterial activities include; iturin, fengycin and kurstakin (Jemil *et al*., 2019). In addition, the *Enterobacte*r species secondary metabolites have been used in the conversion of carbohydrate compounds in fermentation of ethanol and other fermentation products like the process of acid hydrolysate of hemicellulose (Amoah *et al*., 2019). These studies have therefore increased the research on various *Enterobacter* species as industrial microorganisms.

2.3 The Genus *Paenibacillus*

Paenibacillus are Gram negative or Gram variable rod-shaped bacillus. They are motile, anaerobes and form endospores slightly larger than its vegetative form. The species in this genus were initially placed in the genus *Bacillus*. The name *Paenibacillus* can be broken into two words i.e. paene (latin adverb which means almost) and *Bacillus*, hence the name almost a bacillus (Grady *et al*., 2016) (*Paenibacillus*). The genus *Paenibacillus* is found in the family *Paenibacillaceae*. The genus is currently comprised of about 200 species. *Paenibacillus* are mostly found in different types of soils associated with roots.

Many *Paenibacillus* strains are known to have ability to hydrolyze cellulose (cellulolytic *Paenibacillus*), hemicellulose and lignin (Yadav and Dubey, 2018). Others are involved in the degradation of various textile dyes, diesel fuel, polyvinyl alcohol, bitumen, benzene and other compounds (Grady *et al*., 2016). Despite having different application in pharmaceutical and manufacturing industries, varieties of *Paenibacillus* are responsible for the opportunistic infections of humans while others cause spoilage of food.

2.3.1 Antimicrobial properties of *Paenibacillus*

Paenibacillus produce various antimicrobial compounds hence able to rival other microorganisms. A case study of 25 *Paenibacillus* isolates from water and soil samples was done and 15 isolates were found to have a wide inhibition spectrum against tested pathogenic bacteria and fungi (Zhai *et al*., 2021). This suggested that *Paenibacillus species* are promising producer of antimicrobial products (Grady *et al.,* 2016). The antimicrobial products of *Paenibacillus* include peptide, volatile organic compounds (VOC) and enzymes with potential applications in pharmaceutical and food processing (Mülner *et al*., 2021). *Paenibacillus* produce two types antimicrobial peptides i.e. ribosomally synthesized bacteriocins (lantibiotics and pediocins) and non- ribosomally synthesized peptides. Lantibiotics contain amino acid lanthionine which has activity against Gram-positive bacteria and some Gram negative bacteria (Huang 2015). Few studies have been done on pediocins compared to lantibiotics (Van Heel *et al*., 2016).

Paenibacillus produce mainly non-ribosome synthesized peptides. They act by disrupting membrane of target cells and since the target organisms do not recognize their membrane easily the development of resistance is reduced (Pajor *et al*., 2020). There are three known categories of non-ribosomal lipopeptides i.e., cyclic cationic, cyclic non cationic and linear cationic. Limited research has been done on linear cationic non ribosomal lipopeptides.

The *Paenibacillus* also produce useful molecules such as exopolysaccharides, enzymes such as amylase, cellulases, lipases, pectinase, hemicellulase, lignin modifying enzymes, oxygenases, mutanases, and dehydrogenases. These molecules have applications in detergents, food, textiles, biofuel and paper manufacturing industries (Glady *et al*., 2016).

2.3.2 Biochemical, morphological and physiological characteristics of *Paenibacillus*

Colonies are regular, small (3mm in diameter) and grey in color. Cells are Gram- negative or Gram variable rods that are about 1µm wide and 3-5 µm long. *Paenibacillus* cells appear singly, paired, or as short chains (Priest, 2015).

Most *Paenibacillus* species are negative for oxidase and catalase tests, grow at a temperature range of 30°C and 37°C and 2% NaCl media. *Paenibacillus* do not grow in nutrient broth. Most strains are positive for acid production from starch and glucose, degradation of gelatin and casein but do not hydrolyze starch (Kumar *et al*., 2018).

2.4 The Genus *Brevibacillus*

The genus *Brevibacillus* belong to the family of *Paenibacillaceae* and was initially recognized as *Bacillus brevis*. *Brevibacillus* are Gram-positive or Gram variable aerobic bacteria (Yang and Yousef, 2018). Their cells are rod-shaped, round-ended and usually occur singly, in pairs or in chains. The cell size range from 0.7- 1.0 µm in diameter and 3.0- 6.0 µm long. They bear endospores which are found in sporangia. Their colonies are smooth and light orange colored. The DNA G + C content of *Brevibacillus* ranges from 40.2 to 57.4 mol %. Under this genus more than twenty species have been discovered so far. *Brevibacillus* inhabit diverse environments and it is among the most distributed genera of Gram-positive bacteria (Yang *et al*., 2016). They are the best laboratory models due to their rich growth and

protease production. The enzymes produced by this genus have application in biocontrol due to their ability to suppress different human and plant pathogens (Ruiu, 2020).

2.4.1 Morphological, physiological and biochemical characteristics of genus *Brevibacillus*

Most of *Brevibacillus* are Gram positive or Gram variable, aerobic, spore forming and motile bacilli. They grow best at pH 5.5- 5.7 and temperature of between 28ºC -30º C. They are oxidase negative, catalase positive, indole negative and reduce nitrate to nitrite. *Brevibacillus* utilize citrate and ammonium compounds and produce acid from the fermentation of D-glucose. They are able to hydrolyze casein, gelatin, DNA and tween 60 (De Oliveira *et al.*, 2020; Meena *et al.*, 2014).

2.4.2 Biocontrol of *Brevibacillus*

Brevibacillus species produce antibiotic gramicidin S with activities against lipid bilayer of membrane of other microorganisms (Ray, 2020). Others have been shown to have antifungal properties e.g. *Brevibacillus brevis* produce bioactive substances against *Fusarium wilt* of pigeon pea (Jiang *et al.*, 2015). Gramicidin S produced by *Brevibacillus brevis* also has sporicidal effects against conidia of *B. cinara* (Wenzel *et al.*, 2018). *Brevibacillus brevis*, *B. formosus* and *B. agri* produce various inhibitory metabolites e.g. siderophore, hydrogen cyanide (HCN) and chitinase that suppress root rot diseases and *Salvia officinalis* wilt (Omar and Ahmed 2014). *B. brevis* XDH produced a linear peptide tostadin that demonstrated significant antimicrobial activities against *S. aureus* and *E. coli*. *B. laterosporus* that has no history of pathogenicity to humans and animals was found to produce antimicrobial compounds that were active against Gram-positive bacteria (Odah *et al.*, 2020). The Brevibacillin that is produced by *B. laterosporus* has strong antibacterial activity against *S. aureus* and Vancomycin resistant *Enterococcus faecalis*. Brevibacillin also has antimicrobial activity against *Clostridium difficile* indicating its potential antimicrobial activity against

anaerobic microorganisms. Members of *B. laterosporus* are also known to produce anti-tumor agents and thrombin inhibitors (Fathizadeh *et al*., 2021). Laterosporulin which was named after the characterization of class II d bacteriocin from *B. laterosporus* strain GI-9, displays broad-spectrum antibacterial activities through membrane permeabilization (Ning *et al*., 2021; Baindara, *et al*., 2016).

2.5 Antimicrobial resistance

The tendency of microorganisms to resist antibiotics treatments and therapies is referred to as antimicrobial resistance. Increasing antimicrobial resistance has presented a major threat to public Health since it lowers the ability of antibiotics leading to increase in morbidity and mortality rate (Dadgostar, 2019). For instance, in 2001, ceftriaxone-resistant *Salmonella typhi* was reported in Bangladesh. In 2016 there was an outbreak of *Salmonella typhi* in Pakistan which gained resistance to antibiotics like chloramphenicol, cotrimoxazole, streptomycin, ampicillin, cephalosporins and fluoroquinolones (Hooda *et al*., 2019). Vancomycin that has been used to treat methicillin resistant *Staphylococcus aureus* for many years has gained resistance against clinically isolated *S. aureus* in recent years (Cong and Yang, 2020).

The uncontrolled use of antibiotics has contributed to the increase in the development of antimicrobial resistance. The main factors responsible for misuse of antibiotics include; over the counter availability of antibiotics without professional prescriptions, use of low potent and effective drugs and the availability of drugs from hawkers who have little knowledge about drugs indications and contraindications (Abdou *et al*., 2021).

Antimicrobial resistance is a global problem. Globalization has increased the chances of countries to import infectious diseases. Many strategies have been put in place to combat

antimicrobial resistance. These strategies aim at avoiding the emergence of new resistance and the prevention of the transmission of already existing resistance (Freeland *et al*., 2021).

The mostly applied strategies globally include; surveillance of antimicrobial resistance, the tracking of antibiotics consumption, encouraging the research and development of new antimicrobial as well as adoption of measures that ensure the rational and appropriate use of existing antimicrobials (Abushaheen *et al*., 2020).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of the study area

Lake Magadi is a hypersaline- alkaline soda lake located near Kenya-Tanzanian border and covers an area of about 100 square kilometers (Figure 1). The lake is sited at an altitude of 600 meters above sea level and coordinates of 1° 52' 59" South, 36° 16' 0" East. The area has an average rainfall amount of about 500mm per annum. Lake Magadi has alkalinity range of 9-12, salinity of upto 35% w/v or greater and a temperature range between 22° C -34° C. It has been referred to as alkaline saline pan due its huge accumulations of solid Na_2CO_3, NaCl and trona ($NaHCO_3.Na_2CO_3.12H_2O$) and also high rates of up to 3500mm per year during drought (Kambura *et al*., 2016). Despite the study showing that Lake Magadi ecology is rich in biodiversity particularly microorganisms, less researches have previously been conducted.

Lake Bogoria is also an alkaline, saline soda lake located in the Kenyan Rift Valley and is found on coordinates of 0" 20'N and 36" 15'E (Figure 1). The lake is located south of Lake Baringo with a salt concentration of 5% w/v and a pH of 9.0. It has atmospheric temperature of 37^oC while the stream waterway and hot spring water temperatures are 76^oC and 90 ^oC respectively. Lake Bogoria is about 10 meters deep, 34 km long and a width of 3.5 km, with a drainage basin of about 700 km^2 and an altitude of 990 m. The lake gets inflow from Rivers Emsos and Sandai and from the hot springs Chemurkeu, Loburu and southern group southern group (Losaramat and Ng'wasis, Koibobei) (Salano *et al*., 2017).

Lake Elementaita is an alkaline soda lake located at 0º 27'S, 36° 15'E on the Kenyan Rift valley with an altitude of 1776 meters above sea level (Figure 1). Lake Elementaita covers an area of about

g630km^2 and is fed by two rivers, i.e. River Mbaruk from Northern part and River Kariandusi from Eastern plateaus. Lake Elementaita basin occupies an area of about 630km^2 (Ondiere *et al*., 2017).

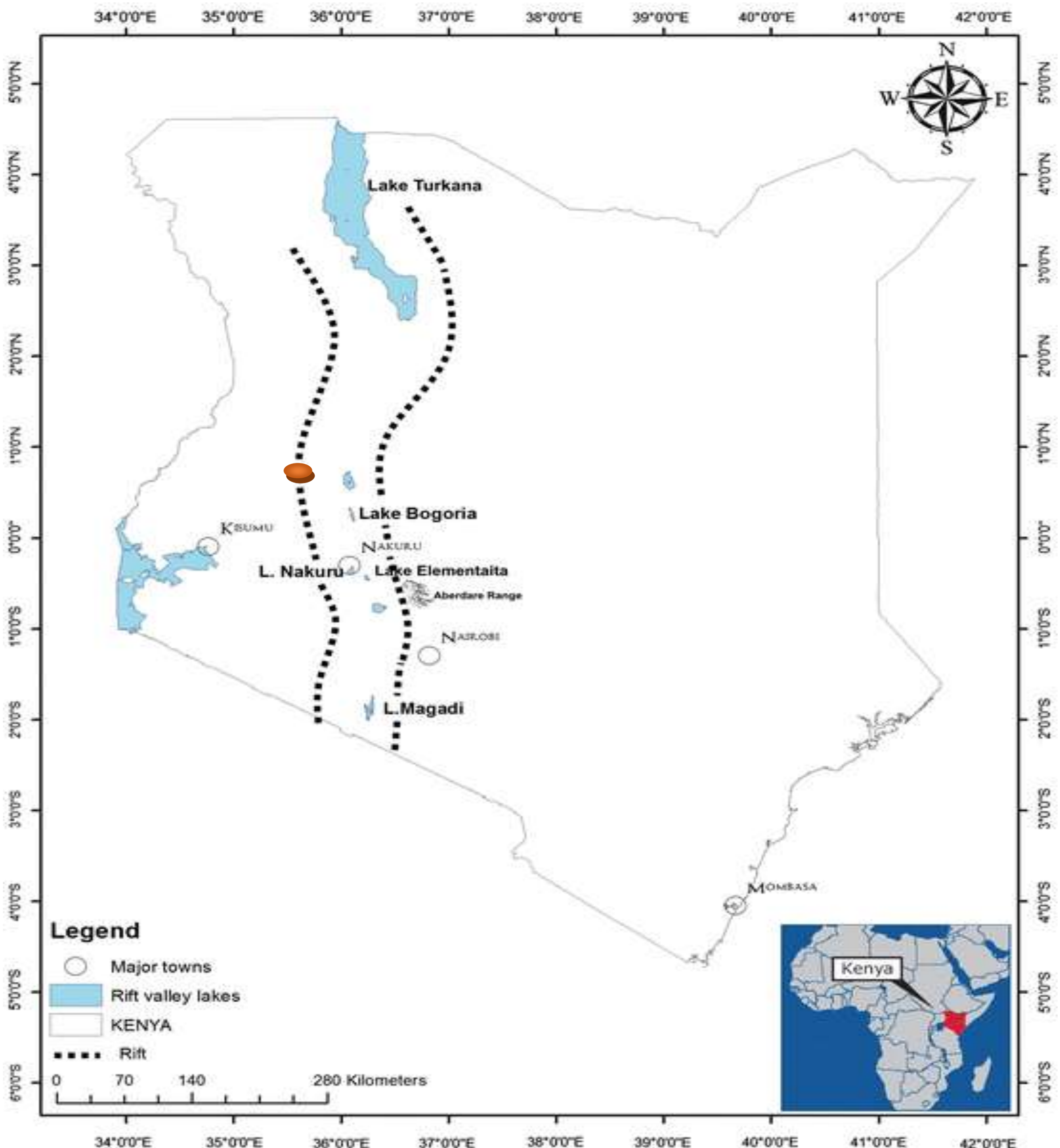

Figure 1: Map of Kenya showing soda lakes of the Rift Valley (Kenya) Springer link

3.2 Study design and sampling

Four sampling sites were identified in each sampling area of Lakes Magadi, Bogoria and Elementaita. Simple random sampling design was used. In each sampling site, two samples (10 g each) of sediments was randomly collected from the upper few centimeters (5 cm) of the soil profile by help of sterilized soil sampling auger. A total of 24 samples (8 samples

from each sampling area) were collected. The samples were spread on a clean polythene paper and foreign matters such as stones and leaves were removed and air-dried to reduce the formation of the molds. They were then packed in sterile polythene bag and labeled. The label contained details about name of the sampling site, sampling area and the sampling date. Samples were packed in a cool box and aseptically transported within 24 hours to the Biochemistry Laboratory of the University of Nairobi, Chiromo Campus for analysis. The samples were stored at 4^oC in the cold room prior to the analysis**.**

3.3 Isolation procedures

The isolation of *Enterobacter, Paenibacillus* and *Brevibacillus* was done using two types of media i.e. a modified Stan 21 agar and water agar (Appendix A). Preparation of both media was done in a manner that preserved the soda lake conditions of mineral conditions, salinity, and pH. The modified Stan 21 agar consisted of solution A (1g K_2HPO_4, 0.02g yeast extract and 16g Agar in 700 ml of distilled water) and solution B (1g KNO_3, 1g $MgSO_4.7H_2O$, 1g $CaCl_2.H_2O$, 0.2g $FeCl_3$, 0.1 g $MnSO_4.7H_2O$ in 300ml of distilled water). Solution A was heated to boiling to dissolve the content then both solutions were autoclaved separately at 15lbs and 121 ^oC for 15 minutes. The two solutions were mixed and allowed to cool to 50 ^oC. The media was supplemented with 10ml of 100mg/ml of streptomycin and poured into sterile petri dishes. The streptomycin would inhibit the growth of fast growing bacteria. Therefore, by the time the bacteria species of genera *Enterobacter, Paenibacillus* and *Brevibacillus* start growing (3rd day of incubation), the contaminants will have been eliminated. After solidification, sterile Whatman No.1 filter paper ($2cm^2$) (source of cellulose) were placed at the center of the petri dishes. About 0.3g of the soil samples were inoculated on the Whatman filter paper using sterile forceps. The plates were then incubated at 30^oC for two to three weeks.

The bacteria were also isolated using baiting technique. *Escherichia coli* strain 25922 was used as the bait. The *E. coli* was sub cultured in test tube containing 15 ml of Luria broth (prepared by mixing 10g/L Tryptone, 10g NaCl and 5g Yeast extract then autoclaved at 121 ^{o}C and 15lbs for 15 minutes) for 24 hours at 37 ^{o}C. After 24 hours the centrifugation of *E. coli* suspension was carried out at 10,000 rpm for 10 minutes. The bacteria pellets were re suspended with 100mg/ml streptomycin solution, to make a thick suspension of bacteria cells. Water medium was prepared by putting 1.5g $CaCl_2$, 1.5g Mg SO_4, 11.9g Hepes and 15g Agar in 1000ml of distilled water then heated to boiling to dissolve contents. It was then autoclaved at 15lbs at 121 ^{o}C for 15 minutes. The medium was supplemented with 10 ml of 100mg/ml streptomycin solution. The dead *E. coli* cells were streaked on the water agar medium using sterile swabs. Soil samples were inoculated at the center of the cross streaks or at the end of each parallel streaks by use of a sterile swabs. The petri dishes were incubated at 30 ^{o}C for two to four weeks.

3.3.1 Sub culturing and purification

The plates with positive growth were sub cultured in CY and VY media. VY medium was prepared by dissolving 5 g of baker's yeast, 1g $CaCl_2.2H_2O$ and 15g Agar in 100ml of distilled water whereas, CY medium was prepared by dissolving 3g casein, 1g yeast extract, 1g $CaCL_2$, 10g Hepes, and 16g Agar in 1000ml of distilled water. The pH of both media was adjusted to pH 7.0-7.2 using HANNA pH meter model H1 2211 pH/ORP. To decrease the pH 1M NaOH solution was added dropwise with regular stirring until it reached pH 7.0. The media were then boiled to dissolve then autoclaved at 121°C and 15lbs for 15 minutes. The media were poured into plates inside the biosafety cabinets then allowed to solidify. A piece of swarm or fruiting body were picked using sterile sharp point of a disposable injection

syringe and inoculated on the surface of CY and VY media at separate locations. The petri dishes were incubated at 30°C and observed daily for three weeks.

3.4 Morphological characterization of the isolates

Morphological characterization of isolate involved the observation of colonies of the cultures. Features like colony shape, color, texture and the formation of the fruiting bodies were observed. Gram staining was done using crystal violet and safranin stains to differentiate between Gram negative and positive bacteria. The slides were observed under compound microscope at magnification of X100. The shape and characteristics of the cell were also observed and recorded.

3.5 Biochemical characterization

3.5.1 Catalase test

This test was done by picking the colony of 18 to 24 hours old culture and placing it in a test tube containing 2ml 3 % hydrogen peroxide solution. Positive results were indicated by the Production of bubbles while negative results were indicated by the absence of the bubbles.

3.5.2 Oxidase test

The test was performed by picking colonies from a fresh (24 hours old culture) bacterial plate by help of a sterile wire loop and rubbing it into an oxidase disk. Change of colour to purple within 5 to 10 seconds showed a positive oxidase test while lack of colour change after 2 minutes showed a negative oxidase test. Change of colour within 6 to 90 seconds indicated delayed oxidase positive.

3.5.3 Nitrate reduction test

Nutrient broth (0.8%) was supplemented with 1 % KNO_3, 4 % NaCl and 1 % Na_2CO_3. The isolates were inoculated into the media in duplicates and incubated at 30°C for 48 hours.

After incubation, 0.2 ml alpha- naphthylamine and sulphanilic acid each were added to the 0.5 ml of the cultures. Formation of cherry red colour showed Positive reactions while presence of yellow colour showed negative reactions. Zinc powder was added to confirm the results, whereby the red colour showed negative results while lack of colour change showed that reduction was far off nitrite.

3.5.4. Indole production and hydrogen sulphide production test

The isolates were inoculated using stabbing method in duplicates in sulphur indole motility (SIM) agar then incubated at 37°C for 48 hours. An uninoculated tube was used as control. The Kovac's reagent was added to the 48 hours old culture. Presence of a cherry red layer was an indication of a positive result for indole production while the presence of brown colour indicated a negative result. Presence of black colour indicated the isolates were able to produce H_2S, while the absence of black colour indicated that bacteria were not able to produce H_2S from the media.

3.5.5 Triple Sugar Iron Test

The TSI Agar was prepared by dissolving 65 grams of medium in 1liter of distilled water. The medium was put in a test tube and autoclaved at 121°C and 15lbs pressure for 15 minutes. It was then allowed to set in slope and a butt of 2.5-inch long. Using inoculating needle, the inoculum from the isolates were stabbed into the medium and streaked on the surface of the medium. The cultures were incubated at 37°C for 24 hours. Glucose utilization was indicated by acid production on the butt (the colour change from red to yellow), while acid production in slant (yellow) and butt (yellow) indicated sucrose or lactose fermentation. The formation of bubbles displacement or fracturing of medium indicated the gas production from sugar fermentation.

3.6 Molecular characterization

3.6.1 DNA extraction

The DNA extraction was done using bacteria culture which was grown in CY broth for 5 days at 30ºC. The broth was separated by centrifugation for 15 minutes at 7000rpm. The upper layer of the broth was decanted into an Eppendorf tube and 550 µl of the DNA extraction buffer added to it and mixed by repetitive pipetting. Using a sterile 1000 µl pipette, 530 µl of the mixture was transferred to sterile microfuge tube followed by addition of 60µl of SDS (10%) and 10 µl of proteinase K. The content was centrifuged then incubated in the water bath at 65º C for 30 minutes. After incubation the mixture was removed and allowed to cool for 5 minutes, followed by the addition of 100µl of 5M NaCl. The solution was mixed well until it became homogenous. Eighty microliters of CTAB/ NaCl was added and mixed for 5 minutes then incubated at 65ºC for 10 minutes in the water bath. After incubation the mixture was allowed to cool. Equal amount of Chloroform: Isoamyl alcohol prepared in a ratio of 24:1 was then put into the mixture and the content mixed well by inverting the Eppendorf tubes gently. The mixture was centrifuged at 12000 rpm for 15 minutes. The upper layer of the content was moved into another sterile Eppendorf tube using yellow tips for every sample then topping up with sterile distilled water to get equal volumes of the samples and the step repeated before adding 0.6 by volume isopropanol. The mixture was incubated for 30minutes or I hour at -20 ºC. After incubation, the mixture was tapped gently then centrifuged at 12000rpm for 15 minutes. The orientation of the Eppendorf tube in the centrifuge was noted. The supernatant was poured and the content in the microfuge tube air-dried for 5 minutes followed by addition of 200µl of 70% ethanol into the mixture. The content was centrifuged at 12000rpm for 15 minutes to precipitate the DNA. The supernatant was removed using a pipette to avoid losing the DNA and air dried for 5 minutes. Using a sterile 100 µl pipette, 45µl of sterile distilled water was added and stored at 4ºC overnight so

that the pellets can dissolve. The electrophoresis was run using 1.2 % agarose gel stained with ethidium bromide in 1x TAE buffer at 100V for 1 hour and 30 minutes. The visualization was done under UV trans illuminator.

3.6.2 Polymerase Chain Reaction

The 16S rDNA gene sequence was amplified using a Bio-Rad thermocycler (model PTC-100) and universal bacterial primer pair B27F an d 5'-AGAGTTTGATCMTGGCTCAG-3'and 1492R 5'GGTTACCTTGTTACGACTT-3'. The gradient PCR was performed to optimize the annealing temperature. The PCR mix was made of 2.0 µl of genescript Taq, 2.0 µl (5-pmol) of B27F forward primer, 2.0 µl (5-pmol) of 1492R reverse primer, 2 µl of template DNA, 2.0 ul of dNTPs mix (2.5mM), 5.0 PCR 10x buffer with Mg (genescript), 2.0 µl $MgCl_2$ and 33 µl of PCR water. The initial enzyme activation was done at 94°C for 5 minutes followed by 35 cycles of a denaturation step at 94°C for 1 minute, primer annealing step at 48°C for 1 minute, chain elongation step at 72°C for1 minute and a final extension at 72°C for 5 minutes. The presence of amplified products and their concentration were determined by running 5 µl of product on 1% agarose gel stained with ethidium bromide in 1x TAE buffer at 80V for 1 hour. The visualization was done under UV trans illuminator.

3.6.3 Purification of PCR products

The PCR products obtained were purified using the GFX DNA and Gel Band Purification kit. Using sterile 1000 µl pipette, 500µl of capture buffer type 3 was added to the sample. GFX microspin column was placed into collection tubes for each purification. Capture buffer type 3 sample mix was briefly centrifuged to obtain the solution at the bottom of the tube. The Capture buffer type 3 sample mix was put onto the assembled GFX microspin column with collection tube and the mixture centrifuged at 16000g for 30 seconds. The collection tube was emptied to discard the flow. The GFX microspin column was put back into the collection tube. The wash buffer type 1 (500µl) was put into the GFX microspin column and the content

centrifuged at 16000gfor 30 seconds. The collection tube was removed and GFX microspin fixed into DNase free 1.5ml Elution buffer type 6 (35µl) was added to the assembled GFX microspin column and. The assembled GFX microspin column and Eppendorf tube was at room temperature for 1 minute followed by centrifugation at 16000g for 1 minute to obtain the purified DNA.

3.6.4 Sequencing and phylogenetic analysis

Purified samples were run on agarose gel to determine the concentrations and check the quality of the PCR products. The products together with the primers were then labeled and packed in ice packs and shipped through FedEx shipping company to Macrogen Europe, Netherlands for sequencing. The gene sequences of the isolates were compared with the public data sequences, using the Basic Local Alignment Search tool (BLAST) obtained from the National Centre for Biotechnology Information (NCBI) website (htt://www.ncbi.nih.gov). The alignment of the sequences was done using MUSCLE v3.8 by retrieving and adding to the database the 16S rDNA gene sequences which were more similar to those determined in the study and aligning them with the MUSCLE (Oliveira *et al*., 2016). BioEdit software was used in correction of the alignments based on conserved regions using (Irshad *et al*., 2021). The evolutionary relationship of these taxa was inferred using the Bayesian inference of phylogeny method (Meyer *et al*., 2019) by Mr Bayes software v 3.2.7 (Ayres et *al*., 2019). Trees were constructed using the generalized time reversal (GTR) nucleotide substitution model in which a proportion of sites have invariable distribution and rate matrices and stationary state frequencies have a fixed compound dirichlet distribution. Shape parameters have a uniform distribution and all branch lengths have a compound gamma dirichlet distribution. Two separate runs (run 1 and 2) comprising four chains (three "heated" chains and one "cold" chain) were executed by Mr Bayes using MCMC (Markov Chain Monte Carlo) sampling over 12, 0000, 0000 generations at a sample frequency of 1000. The first

25% of trees sampled were discarded during posterior probability estimation of the trees. After 12,000,000 generations, the analysis was discontinued when the average variance of branched frequencies approached 0.0. The optimal trees drawn were visualized by Figtree software v1.4.4. Trees were drawn to scale, with the lengths of the branch in the same units as those of the evolutionary distances used to derive the phylogenetic tree and were in the units of the number of base substitutions per site.

3.7 Fermentation and crude extract preparation

Isolates were fermented in conical flasks containing 100ml of CY broth. The medium was weighed and placed in a conical flasks followed by addition of 4g of Amberlite XAD 16 absorbent resin. The mixture was autoclaved at 120°C and 15 lbs for 15 minutes. The bacteria isolates were inoculated into the broth and incubated at 37°C for 7 days. Two flasks were not inoculated with any bacteria and were therefore treated as control test. After incubation the resin was filtered and washed three times using methanol. The methanol soluble fractions were concentrated using a rotary evaporator and a dark brown viscous extract was obtained. Approximately 0.25g of each crude extract was dissolved in 10ml of methanol resulting in crude extracts of 2.5mg/100ml concentration.

3.8 Antimicrobial activity of the isolates

Antibacterial activity of the crude extracts from the bacteria isolates was determined by agar well diffusion method. Inoculation containing approximately 100 cfu/µl of the test pathogenic bacteria were inoculated on Muller Hinton agar plates using sterile swabs. Using sterile blue micropipette tips, wells of 8 mm diameter were made in the Muller Hinton agar medium. One drop of molten Muller Hinton agar was added to the wells using a sterile pipette to seal up the bottom of the wells. Each well was filled with 100µl (25mg/ml) of the crude extract using a sterile pipette. Broad spectrum antibiotic ciprofloxacin CIP) was used as

a positive control. The extracts were allowed to penetrate the medium at room temperature for 1 hour. The cultures were then incubated in the upright position at 37ºC for 18-24 hours. After incubation of the cultures, the diameters size of the zones of inhibition were measured in mm and recorded. The zones of inhibitions of the isolates were compared with the zones of inhibitions of the control. Three replicates of the extract were prepared for each test bacteria. Data were represented as mean of zones of inhibition.

3.9 Statistical analysis

The statistical data for the association between source and the presence of the *Enterobacter, Paenibacillus* and *Brevibacillus* and the association between positive samples and the media used was analyzed using Chi-square test of association. The statistics data for the morphological and biochemical characterization was analyzed using percentages. The statistical data of zones of inhibition was analyzed using Kruskal- wallis test SAS 9.4 software. The evolutionary history was inferred using the Bayesian inference of phylogeny method by MrBayes software v 3.2.7. Trees were constructed using the generalized time reversal (GTR) nucleotide substitution model.

CHAPTER FOUR
RESULTS

4.1 Isolation of bacteria from Lakes Magadi, Bogoria and Elementaita soil samples

Colony formation on Stan 21 agar plates started after the 3rd day of incubation. The colonies developed on the surface and afterwards moved inside the media. The cellulose papers on Stan 21 agar were degraded by the isolates and appeared brown or yellow in color (Figure 2a). Clearing zones on water agar were noted on the 5th day and the growth progressed up to 21st day of incubation. There was appearance of swarms or fruiting bodies along the streaks of the *E. coli* (Figure 2b). In both media the colonies were yellow or orange in color while the fruiting bodies ranged from yellow to brown in color.

Table 1: Isolation on Stan 21 agar

Samples	Source	culture plates (replicates)	Cultures showing bacteria growth
FU103	L. Magadi	2	2
FU 70	L. Magadi	2	2
FU 100	L. Magadi	2	2
FU 79	L. Magadi	2	2
FU 104	L. Magadi	2	1
MC dung	L. Magadi	2	2
FU 78	L. Magadi	2	2
FU 75	L. Magadi	2	2
0.56, D_2 8.3	L. Bogoria	2	2
Soil 9.3	L. Bogoria	2	-
Soil 9.2	L. Bogoria	2	-
Well 2.2	L. Bogoria	2	2
Well 2.3	L. Bogoria	2	-
Site 6.2	L. Bogoria	2	-
Site 6.1	L. Bogoria	2	-
BC dung	L. Bogoria	2	2
$MP_{11}S_1$	L. Elementaita	2	1
$MP_{11}S_1$	L. Elementaita	2	-
$ELHPS_1$	L. Elementaita	2	-
$MP_{11}3$	L. Elementaita	2	-
$MP_{11}S_3$	L. Elementaita	2	1
$MP_{11}S_1$	L. Elementaita	2	1
$ELHPS_3$ H_{13}	L. Elementaita	2	-
BC/5A	L. Elementaita	2	-

Table 2: Isolation on water agar

Samples	Source	culture plates (replicates)	Cultures showing bacteria growth
FU103	L. Magadi	2	2
FU 70	L. Magadi	2	2
FU 100	L. Magadi	2	2
FU 79	L. Magadi	2	2
FU 104	L. Magadi	2	2
MC dung	L. Magadi	2	2
FU 78	L. Magadi	2	-
FU 75	L. Magadi	2	1
0.56, D_2 8.3	L. Bogoria	2	2
Soil 9.3	L. Bogoria	2	2
Soil 9.2	L. Bogoria	2	2
Well 2.2	L. Bogoria	2	2
Well 2.3	L. Bogoria	2	-
Site 6.2	L. Bogoria	2	2
Site 6.1	L. Bogoria	2	-
BC dung	L. Bogoria	2	2
$MP_{11}S_1$	L. Elementaita	2	1
$MP_{11}S_1$	L. Elementaita	2	-
$ELHPS_1$	L. Elementaita	2	-
$MP_{11}3$	L. Elementaita	2	-
$MP_{11}S_3$	L. Elementaita	2	-
$MP_{11}S_1$	L. Elementaita	2	-
$ELHPS_3$ H_{13}	L. Elementaita	2	-
BC/5A	L. Elementaita	2	-

There was no significant ($P > 0.005$) association between media used and bacteria growth, X_2 (1, 100) = 0.6889. However, there were slightly more bacteria growth in Stan 21 agar than in water agar as shown in the Table 3.

Table 3: Association between positive samples and media used in isolation

Media used	Bacteria growth(%)	No bacteria growth (%)	Total(%)
Stan 21 Agar	25	25	50
Water Agar	23	27	50
Total	48	52	100

There was significant ($P < 0.05$) difference between the source and the bacteria growth. Lake Bogoria had higher percentage of bacteria growth (31%) compared to Lakes Magadi (30%) and Elementaita (7%) as shown in Table 4.

Table 4: Association between source and bacteria growth

Bacteria growth	L. Bogoria (%)	L. Elementaita (%)	L. Magadi (%)	Total (%)
Absence	21	25	2	48
Present	31	7	30	68
Total	52	32	32	116

A total of 24 pure isolates were obtained after sub culturing on CY and VY media. The isolates were FU103BB, FU70CC, FU103CA, FU103CB, FU70C, MC dung, FU70BA, FU70CB, FU 75CB, FU70CA, FU103BA, FU70BC, FU75CA, FU104C, FU 70BB, BC dung, FU 78 CB, $MP_{11}S_1C$, BC/5 BC, $MP_{12}3B$, $MP_{11}S_3B$, $MP_{11}S1B$, $ELHPS_3H_{13}$ and BC/5AC

4.2 Morphological characterization of the isolates

The color of the colonies was cream, white, grey or green in pigmentation with percentage proportions of 20.8%, 66.7%, 8.3% and 4.2% respectively (Figure 3). The microscopic observations proved that 75% of the isolates were Gram-negative with 25% being Gram positive (Table 5). The cell shape of the isolates was rods.

Table 5: Morphological characteristics of the isolates

S/No.	Isolate	Colony color In CY or VY medium	Cell shape	Gram staining
1	FU 103 BB	Cream	Short rods	Negative
2	FU 70 CC	White	Rod	Negative
3	FU103 CA	Cream	Rod	Negative
4	FU 103 CB	Cream	Rod	Negative
5	FU 70C	White	Short rods	Positive
6	MC dung	White	Rods in chain	Negative
7	FU 70BA	White	Short rods	Negative
8	FU 70 CB	White	Short rods	Positive
9	FU 75 CB	Grey	Round ended rods	Positive
10	FU 70CA	White	Short rods	Negative
11	FU 103 BA	Cream	Short rods	Negative
12	FU 70 BC	White	Short rods	Negative
13	FU75CA	Grey	Round ended rods	Positive
14	FU 104 C	White	Rod with tapering ends	Negative
15	FU 70BB	White	Short rods	Negative
16	BC dung	Green	Short rods	Negative
17	FU 78 CB	Cream	Rod	Negative
18	$MP_{11}S_1C$	White	Elongated rods	Negative
19	BC/5 BC	White	Elongated rods	Positive
20	$MP_{12}3B$	White	Rods	Negative
21	$MP_{11}S_3B$	White	Branching rods	Negative
22	$MP_{11}S1B$	White	Round ended rods	Negative
23	$ELHPS_3H_{13}C$	White	Short rods	Positive
24	BC/5AC	White	Rods	Negative

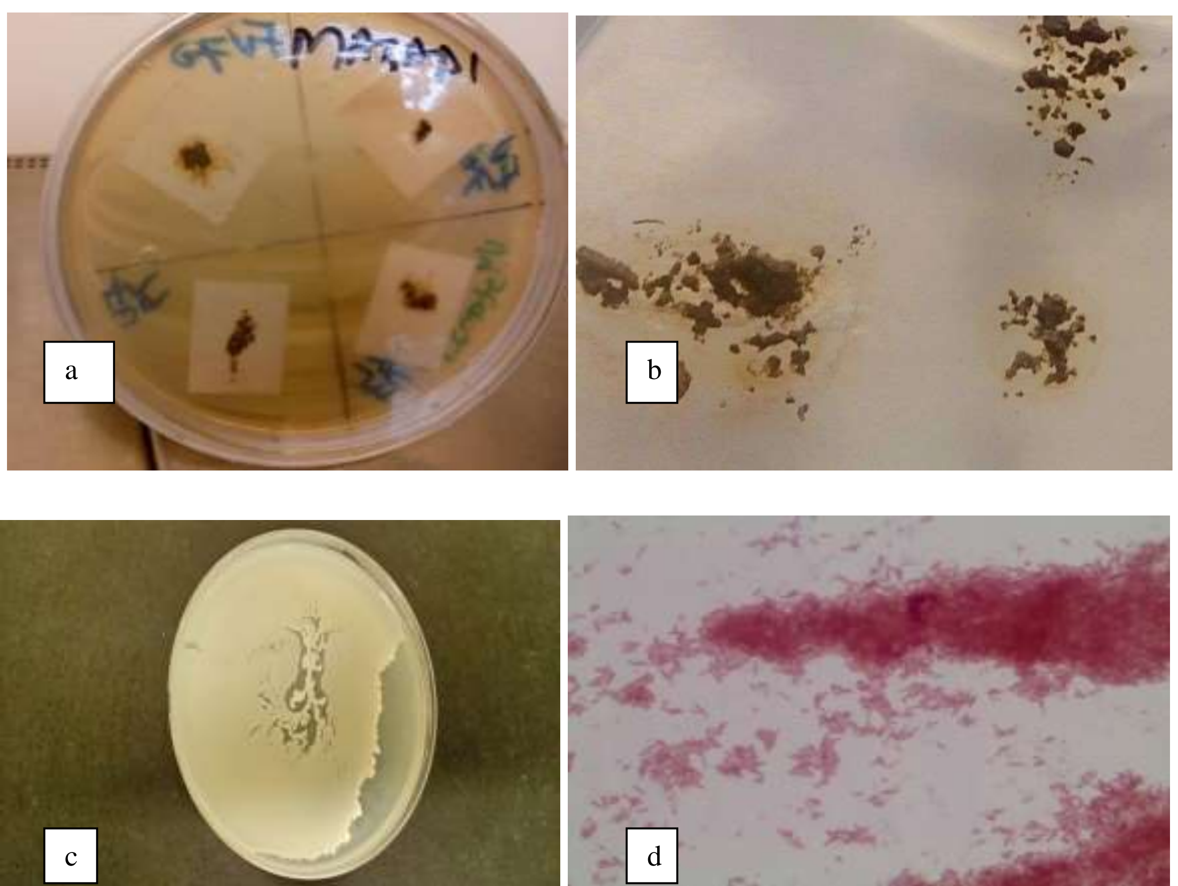

Figure 2: morphological characteristics of isolates

4.3 Biochemical characterization

Out of the 24 isolates, 87.5%, 92% and 58.3% tested positive for nitrate reduction, catalase tests and gas production from the fermentation of glucose respectively while 92%, 87.5% and 96% tested negative for oxidase, indole and H_2S reduction tests respectively (Table 6). Isolates FU75CA and FU75CB were negative for catalase tests and nitrate reduction tests while FU70CC and BC/5AC were positive for oxidase test. Glucose fermentation with the release of gas was observed in all isolates except FU75CB, FU70CC, FU75CA and BC/5CA. All isolates reduced H_2S except isolate FU70BA.

Table 6: Biochemical characterization of the isolates

S/No.	Isolate	Nitrate reduction	Catalase test	Oxidase test	Indole test	H_2S	TSI (B/S) Test	CO_2 Gas production
1	FU 103 BB	+	+	-	-	-	+/+	+
2	FU 70 CC	+	+	+	-	-	-/-	-
3	FU103 CA	+	+	-	-	-	+/+	+
4	FU 103 CB	+	+	-	-	-	+/+	+
5	FU 70C	+	+	-	-	-	+/+	-
6	MC dung	+	+	-	-	-	+/+	+
7	FU 70BA	+	+	-	-	+	+/+	+
8	FU 70 CB	+	+	-	-	-	+/+	-
9	FU 75 CB	-	-	-	-	-	-/-	-
10	FU 70CA	+	+	-	+	-	+/+	+
11	FU 103 BA	+	+	-	-	-	+/+	+
12	FU 70 BC	+	+	-	-	-	+/+	+
13	FU75CA	-	-	-	-	-	-/-	-
14	FU 104 C	+	+	-	-	-	+/+	+
15	FU 70BB	+	+	-	+	-	+/+	+
16	BC dung	+	+	-	-	-	+/+	+
17	FU 78 CB	+	+	-	+	-	+/-	-
18	$MP_{11}S_1C$	+	+	-	-	-	+/+	-
19	BC/5 BC	+	+	-	-	-	+/+	-
20	$MP_{12}3B$	+	+	-	-	-	+/+	+
21	$MP_{11}S_3B$	+	+	-	-	-	+/+	+
22	$MP_{11}S1B$	+	+	-	-	-	+/+	+
23	$ELHPS_3H_{13}$	+	+	-	-	-	+/+	-
24	BC/5AC	-	+	+	-	-	-/-	-

4.4 Molecular characterization

The genomic DNA and the purified DNA products formed clear and visible bands on stained agarose gel under UV trans illuminator.

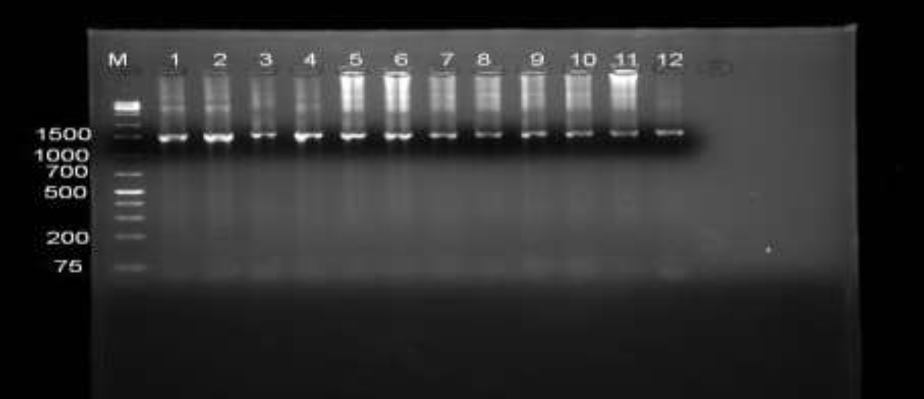

Figure 3: A photograph of PCR amplified 16S rDNA products.

M represent O' Gene Ruler 1kb Plus DNA ladder 75-20000 bp and number 1-12 represents isolates FU103BB, FU 70CC, FU103CA, FU103CB, FU70C, MC dung, FU70BA, FU70CB, FU75CB, FU70CA, FU103BA and FU70BC respectively.

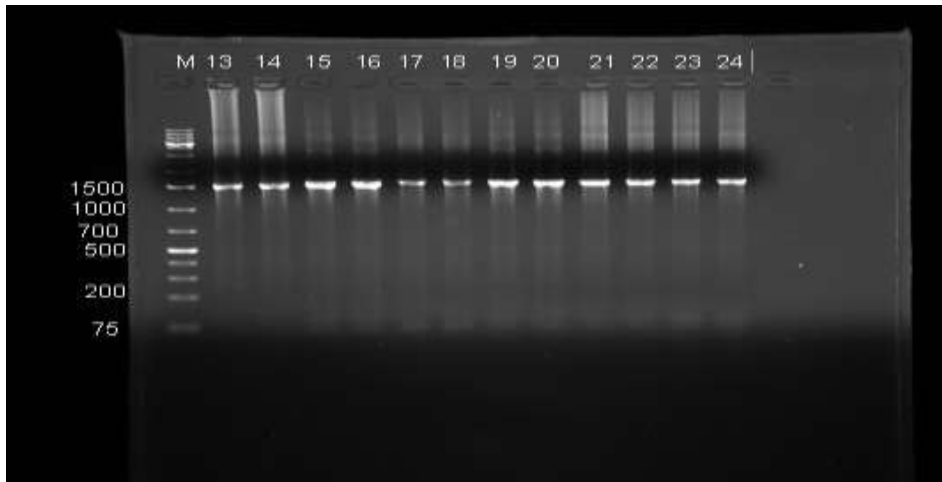

Figure 4: A photograph of PCR amplified 16S rDNA products.

M represent O' Gene Ruler 1kb Plus DNA ladder 75-20000 bp and numbers 13-24 represents isolates FU75CA, FU104C, FU70BB, BC dung, FU78CB, $MP_{11}S_1C$, BC/5BC, $MP_{12}3B$, $MP_{11}S_3B$, $MP_{11}S_1B$, $ELHPS_3H_{13}$, BC/5AC

Sequenced PCR amplicons of the selected isolates were used in the construction of phylogenetic trees. The isolates were found to belong to eight genera according to the Basic Local Alignment Search Tool (BLAST) results. These genera included *Bacillus*,

Enterobacter, *Paenibacillus*, *Brevibacillus* with 4, 8, 2 and 6 species respectively and *Escherichia*, *Providencia*, *Pseudomonas* and *Lysinibacillus* with one species each. The isolates had 95-100% sequence similarity with known members of the same genera (Table 7).

Table 7: BLAST search of the sequenced isolates and their close relatives

S/No.	Isolate	BP	Nearest relatives	Accession number	% similarity
1	FU103 BB	1786	*Bacillus cereus* KII	KF 641827.1	99.65%
2	FU 70CC	1444	*Pseudomonas aeruginosa*	KY549647.1	96.65%
3	FU 103 CA	1455	*Bacillus cereus* MZ1	MWS41635.1	98.95%
4	FU 103 CB	1665	*Bacillus spp*. LQ53	MG025795.1	97.02%
5	FU70C	1494	*Brevibacillus brevis* 513	MN044785.1	96.3%
6	MC dung	1546	*Enterobacter hormaechei* AMS-38	CP051132.5	98.45%
7	FU 70 BA	1715	*Bacillus spp*. CMJ3-7	KCI19112.1	98.66%
8	FU 70CB	1631	*Brevibacillus brevis* AM1-2	MH 938812.1	99.29%
9	FU 75 CB	1490	*Paenibacillus oralis*	MK748165.1	94.26%
10	FU 70CA	1494	*Brevibacillus brevis* 513	MN044785.1	96.3%
11	FU 103 BA	1681	*Enterobacter asburiae* 55MI	MW126534.1	97.50%
12	FU70BC	1577	*Enterobacter asburiae* FDAARGOS	CPO65693.1	96.56%
13	FU 75 CA	1777	*Paenibacillus spp*. BL 16-1	EU912455.1	98.60%
14	FU 104 C	1629	*Enterobacter asburiae* SSMI	MW126534.1	97.56%
15	FU70BB	1426	*Escherichia coli*	MT453882.1	99.22%
16	BC dung	1594	*Enterobacter asburiae* 1808-013	AP019632.1	95.38%
17	FU 78 CB	1830	*Providencia spp*. INS-107	KY964242.1	99.09%
18	$MP_{11}S_1C$	1867	*Brevibacillus brevis* SH37	KC172029.1	99.22%
19	BC/5BC	1803	*Brevibacillus brevis* GE13	KY 312742.1	99.22%
20	$MP_{12}3B$	1578	*Enterobacter asburiae* R7-377	JQ 659874.1	97.53%
21	MP_{11} S_3 B	1722	*Enterobacter spp*.18A 13	APO19634.1	97.65%
22	$MP_{11}S1B$	1612	*Enterobacter spp*. XBGRY7	KJ 1814972.1	99.50%
23	$ELHPS_3H_{13}$	1764	*Brevibacillus brevis* JZY2-35	MT 106877.1	97.75%
24	BC/5AC	1844	*Lysinibacillus fusiformis*	MN911368.1	99.51%

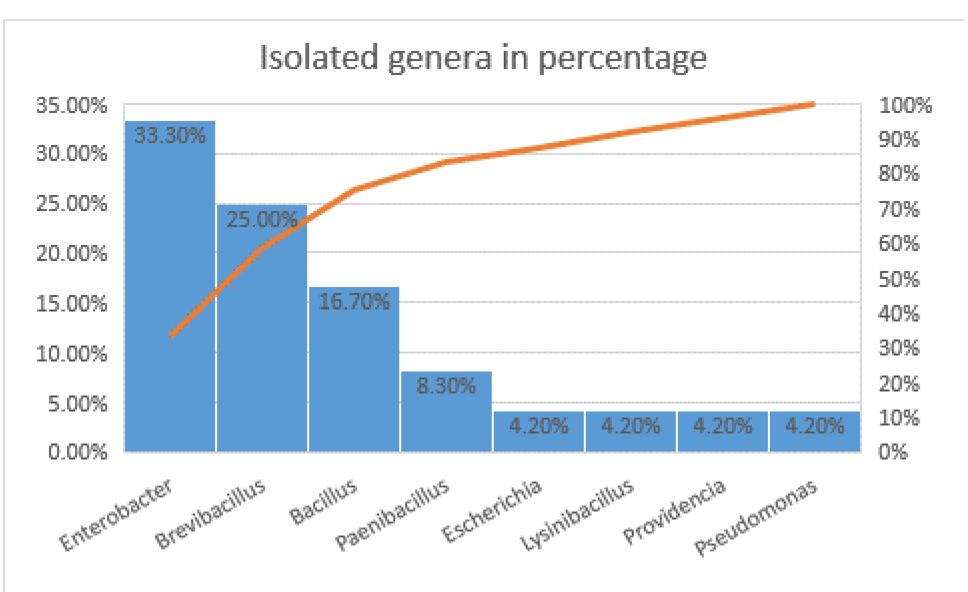

Figure 5: A graph showing the isolated species per genera in percentage

4.5.1 Nucleotide sequence accession numbers

16S rRNA sequences of bacterial isolates 9, 6, 11,14, 16,19 and 23 were deposited to the GenBank via NCBI's Prokaryotic rRNA Submission Portal available at (https://submit.ncbi.nlm.nih.gov/subs/genbank/) under the accession numbers ON619882–ON619888, respectively.

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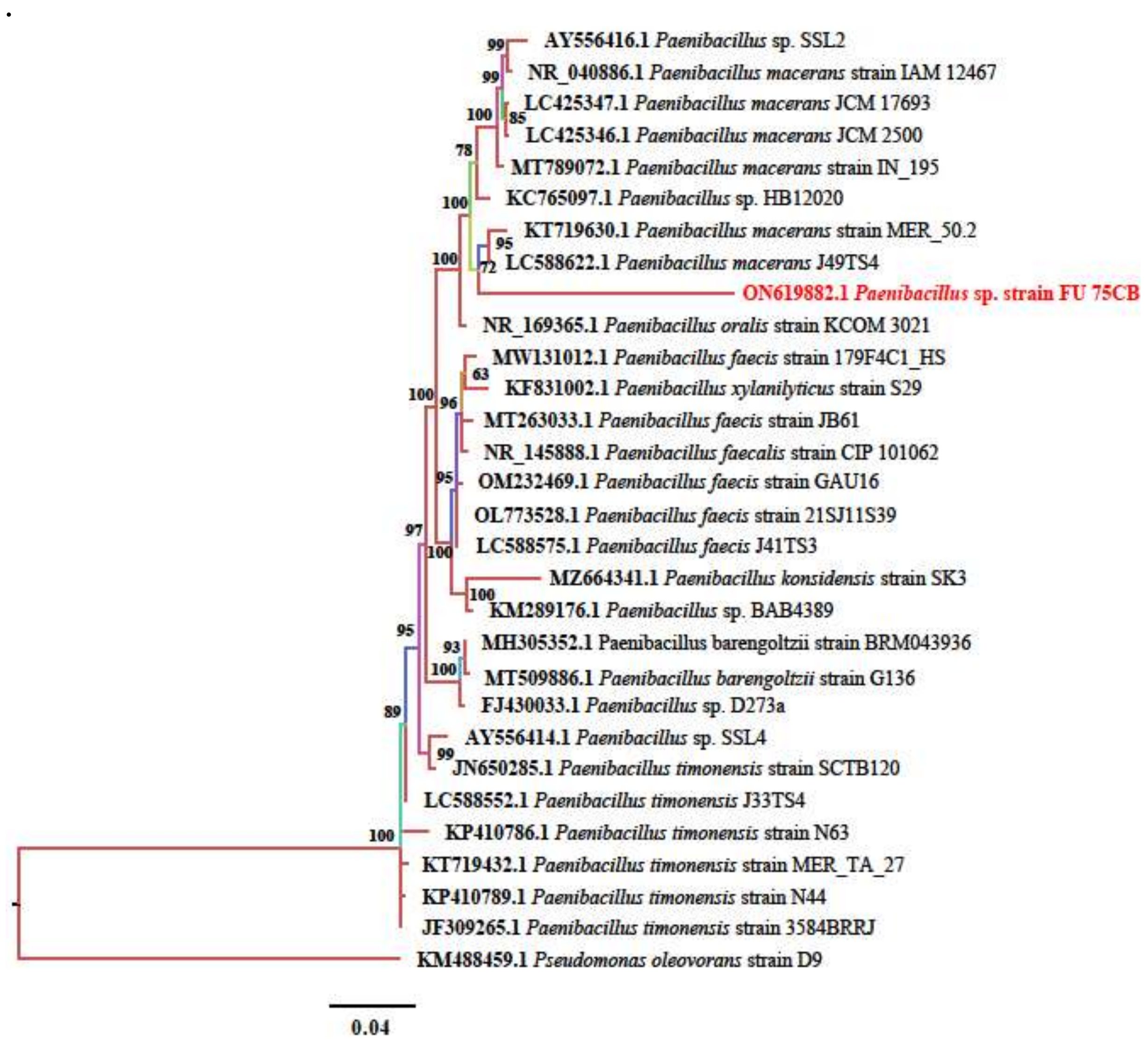

Figure 6: Phylogenetic tree built by Mr Bayes v3.2.7 using twenty-eight 16S rRNA sequences of the genus *Paenibacillus*.

Newly isolated bacterial strain is shown in bold red color (ON619882.1: *Paenibacillus* sp. strain FU 75CB). Numbers shown at the nodes are the percentage posterior probabilities of distribution and scale bar below the tree indicates the number of expected changes (substitutions) per site. KM488459.1: *Pseudomonas oleovorans* strain D9 was used as outgroup in rerooting the tree. Branches are colored based on posterior probabilities.

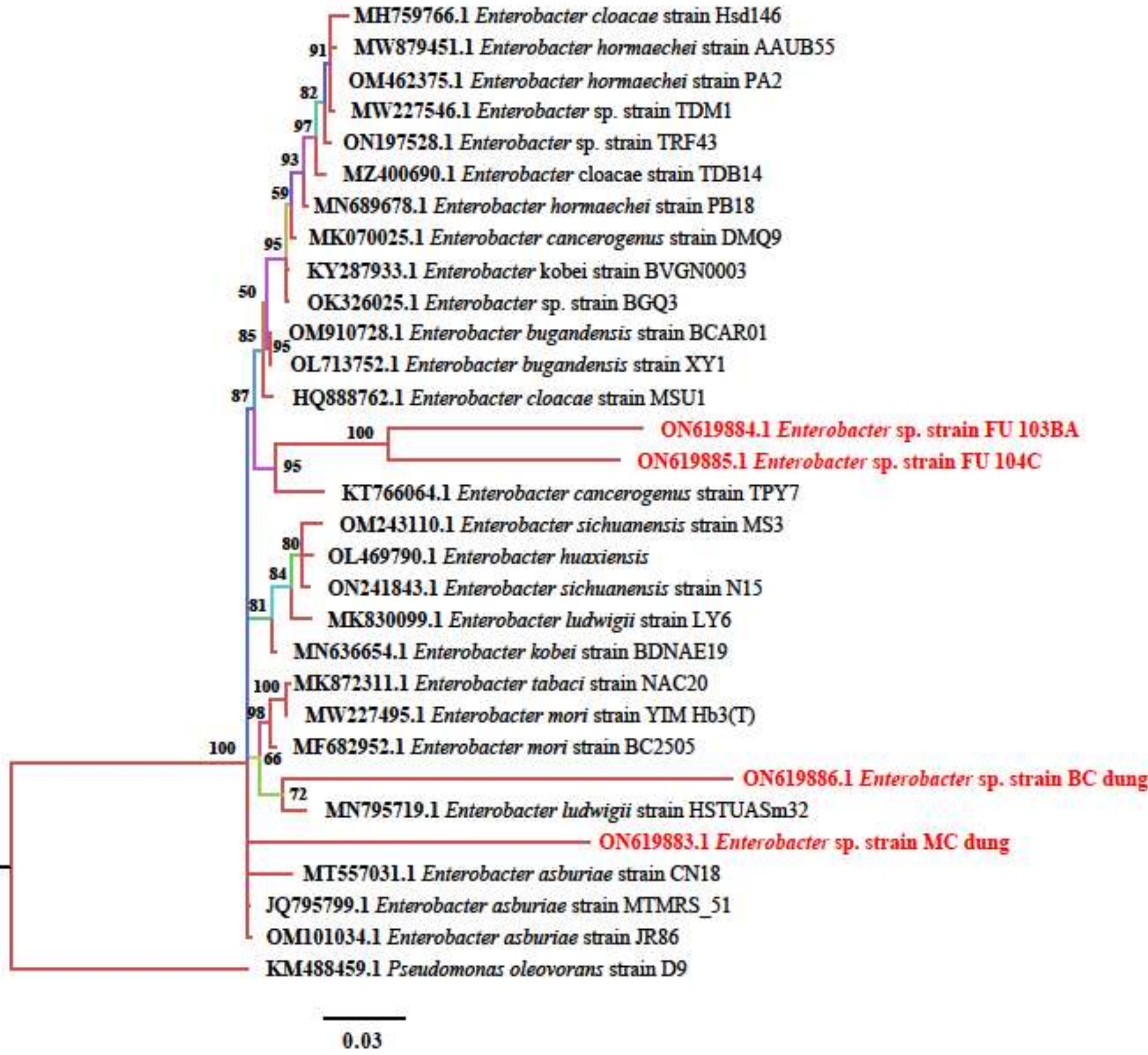

Figure 7: Phylogenetic tree built by Mr Bayes v3.2.7 using twenty-six 16S rRNA sequences of the genus *Enterobacter*.

Newly isolated bacterial strains are shown in bold red color (Accession No. ON619883 – ON619886). Numbers shown at the nodes are the percentage posterior probabilities of distribution and scale bar below the tree indicates the number of expected changes (substitutions) per site. KM488459.1: *Pseudomonas oleovorans* strain D9 was used as outgroup in rerooting the tree. Branches are colored based on percent posterior probabilities.

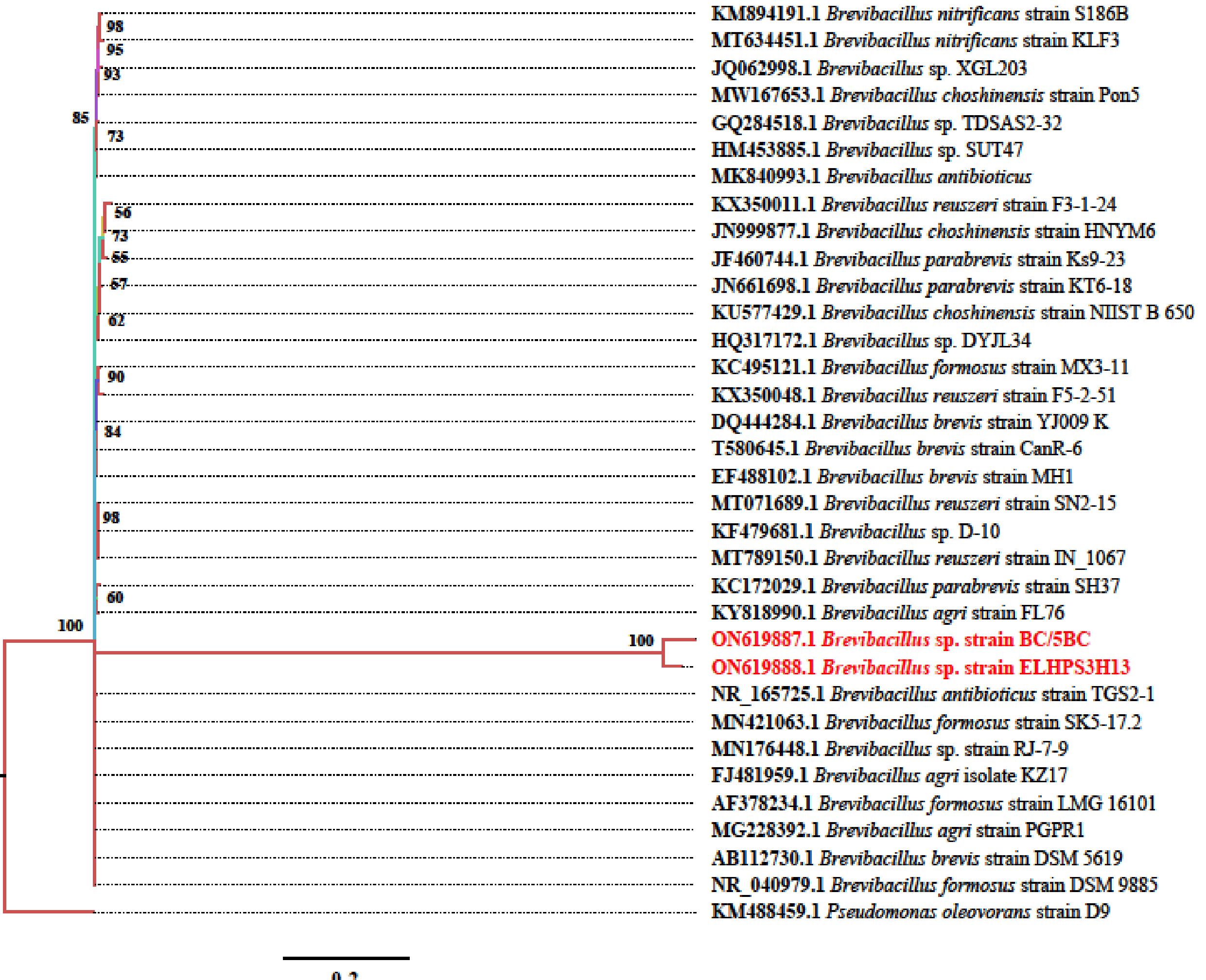

Figure 8: Phylogenetic tree built by Mr Bayes v3.2.7 using thirty-one 16S rRNA sequences of the genus *Brevibacillus*.

Newly isolated bacterial strains are shown in bold red color (Accession No. **ON619887 – ON619888**). Numbers shown at the nodes are the percentage posterior probabilities of distribution and scale bar below the tree indicates the number of expected changes (substitutions) per site. **KM488459.1**: *Pseudomonas oleovorans* strain D9 was used as outgroup in rerooting the tree. Branches are colored based on percent posterior probabilities.

4.6 Antibacterial activity assay of the isolates

Nine out of the twenty-four isolates (*Enterobacter* sp. strain MC dung, *Enterobacter* sp. strain BC dung, *Paenibacillus* sp. strain FU75CB, *Brevibacillus* sp. strain BC/5BC, *Enterobacter* sp. strain FU 103BA, *Enterobacter* sp. strain FU 104C, FU78CB, BC5AC and *Brevibacillus* sp. strain ELHPS$_3$H$_{13}$) expressed antibacterial activity against one or more test pathogenic bacteria i.e. *Salmonella spp*., *E. coli*, *K. pneumoniae*, *Pseudomonas spp*., *S. aureus* and *L. monocytogenes*. The nine isolates belong to genera *Enterobacter*, *Paenibacillus*, *Brevibacillus*, *Lysinibacillus* and *Providencia*. Clear zones of inhibitions were observed after 24 hours of incubation and their diameters were measured in mm. Table 8 shows the most, moderate, least and none active isolates (+ + +, + +, +, and – respectively).

Table 8: Antibacterial activity of the isolates

Isolates	*Salmonella sp*	*E.coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*L. monocytogenes*
MC dung	+ + +	+ +	-	+	+ +	+
BC dung	-	+ +	+ + +	+ +	+ + +	+ + +
FU75CB	+ +	+ +	+ +	+	-	-
BC/5BC	-	+ +	-	-	+	-
ELHPS$_3$H$_{13}$	-	+ + +	-	-	+	+ +
FU 103BA	-	-	-	+	-	+ +
FU104C	-	-	-	+ +	-	-
FU78CB	-	-	+	-	-	+
BC5AC	-	-	+	-	-	-
FU103BB	-	-	-	-	-	-
FU103CB	-	-	-	-	-	-
FU70C	-	-	-	-	-	-
FU70BA	-	-	-	-	-	-
FU70CB	-	-	-	-	-	-
FU70CA	-	-	-	-	-	-
MP$_{11}$S$_3$B	-	-	-	-	-	-
MP$_{12}$3B	-	-	-	-	-	-
MP$_{11}$S$_1$B	-	-	-	-	-	-
FU70BC	-	-	-	-	-	-
FU75CA	-	-	-	-	-	-
FU70BB	-	-	-	-	-	-
MP$_{11}$S$_1$C	-	-	-	-	-	-
FU103CA	-	-	-	-	-	-
FU70CC	-	-	-	-	-	-

Table 9: Activity of the isolates on *Pseudomonas spp*.

Isolates	**N**	**Mean(mm)**	**Median(mm)**	**Minimum**	**Maximum**
BC5AC	3	6.50	0.00	0.00	0.00
B*revibacillus* sp. Strain BC5BC	3	6.50	0.00	0.00	0.00
Enterobacter sp. strain BC dung	3	27.17	16.00	14.00	20.00
Enterobacter sp. strain MC dung	3	24.83	15.00	14.00	15.00
Brevibacillus sp. strain ELHPS3H13	3	6.50	0.00	0.00	0.00
Enterobacter sp. strain FU 103BA	3	12.00	12.00	12.00	12.00
Enterobacter sp. strain FU 104C	3	15.00	15.00	15.00	15.00
Paenibacillus sp. strain FU75CB	3	14.0	11.00	10.00	11.00
FU78CB	3	6.50	0.00	0.00	0.00
Control	3	17.00	11.60	11.50	11.80
H (9)= 28.3784 P=0.0008					

There was significant ($p< 0.05$) difference of antibacterial activity of different isolates against *Pseudomonas spp*. H (9) =28.378, P= 0.0008. Enterobacter sp. strain BC dung had significantly higher inhibition zone (*Md*= 16mm) followed by FU 75 CB and *Enterobacter* sp. strain MC dung both with inhibition zone of (*Md*=15mm). Isolate *Enterobacter* sp. strain BC dung performed better than the control (*Md*=11mm) (Appendix IV a).

Table 10: Activity of the isolates on *Salmonella spp.*

Isolates	**N**	**Mean(mm)**	**Median(mm)**	**Minimum**	**Maximum**
BC5AC	3	11.0	0.00	0.00	0.00
B*revibacillus* sp. Strain BC5BC	3	11.0	0.00	0.00	0.00
Enterobacter sp. strain BC dung	3	11.0	0.00	0.00	0.00
Enterobacter sp. strain MC dung	3	29.0	25.00	25.00	25.00
Brevibacillus sp. strain ELHPS3H13	3	11.0	0.00	0.00	0.00
Enterobacter sp. strain FU 103BA	3	11.0	0.00	0.00	0.00
Enterobacter sp. strain FU 104C	3	11.0	0.00	0.00	0.00
Paenibacillus sp. strain FU75CB	3	26.0	15.00	15.00	15.00
FU78CB	3	11.0	0.00	0.00	0.00
Control	3	23.0	11.00	11.00	11.80
H(9)=28.972 , P= 0.0007					

There was significant ($p < 0.05$) difference of antibacterial activity of different isolates against *Salmonella Spp.* H (9) =28.972, P= 0.0007. *Enterobacter* sp. strain MC dung had significantly higher inhibition zone (*Md*=25mm) followed by *Paenibacillus* sp. Strain FU 75CB (*Md* =15mm). The rest of the isolates had no inhibition zone. Isolate *Enterobacter* sp. strain MC dung performed better than the control (*Md* =11mm) (Appendix IV b).

Table 11: Activity of the isolates on *E. coli*

Isolates	**N**	**Mean(mm)**	**Median(mm)**	**Minimum**	**Maximum**
BC5AC	3	6.50	0.00	0.00	0.00
B*revibacillus* sp. Strain BC5BC	3	17.33	14.00	13.00	15.00
Enterobacter sp. strain BC dung	3	26.17	20.00	18.00	21.00
Enterobacter sp. strain MC dung	3	24.80	18.00	18.00	20.00
Brevibacillus sp. strain $ELHPS_3H_{13}$	3	26.00	25.00	15.00	25.00
Enterobacter sp. strain FU 103BA	3	6.50	0.00	0.00	0.00
Enterobacter sp. strain FU 104C	3	6.50	0.00	0.00	0.00
Paenibacillus sp. strain FU75CB	3	20.67	16.00	15.00	17.00
FU78CB	3	6.50	0.00	0.00	0.00
Control	3	14.00	11.00	11.00	12.00
H(9) = 27.6644, P= 0.0011					

There was significant ($p < 0.05$) difference of antibacterial activity of different isolates against *E. Coli H* (9) =27.664, P= 0.0011. Isolate *Brevibacillus sp.* strain $ELHPS_3H_{13}$ had significantly higher inhibition zone (*Md*= 25mm) followed closely by *Enterobacter* sp. strain BC dung with inhibition zone (*Md* =20mm) (Appendix IV c).

Table 12: Activity of the isolates on *S. aureus*

Isolates	**N**	**Mean(mm)**	**Median(mm)**	**Minimum**	**Maximum**
BC5AC	3	8.00	0.00	0.00	0.00
B*revibacillus* sp. Strain BC5BC	3	19.00	10.00	9.00	11.00
Enterobacter sp. strain BC dung	3	29.00	35.00	35.00	35.00
Enterobacter sp. strain MC dung	3	26.00	24.00	22.00	25.00
Brevibacillus sp. strain ELHPS3H13	3	21.33	11.00	10.00	12.00
Enterobacter sp. strain FU 103BA	3	8.00	0.00	0.00	0.00
Enterobacter sp. strain FU 104C	3	8.00	0.00	0.00	0.00
Paenibacillus sp. strain FU75CB	3	8.00	0.00	0.00	0.00
FU78CB	3	8.00	0.00	0.00	0.00
Control	3	19.67	11.00	1.60	11.00
H(9)= 28.2923, P=0.0009					

There was significant ($p < 0.05$) difference of antibacterial activity of different isolates against *S. aureus, H* (9) =28.292, P= 0.0009. *Enterobacter* sp. strain BC dung had significantly higher inhibition zone (*Md*=35mm) followed by *Enterobacter* sp. strain MC dung (*Md*=24mm) (Appendix IV d).

Table 13: Activity of the isolates on *L. monocytogenes*

Isolates	**N**	**Mean(mm)**	**Median(mm)**	**Minimum**	**Maximum**
BC5AC	3	6.50	0.00	0.00	0.00
B*revibacillus* sp. Strain BC5BC	3	6.50	0.00	0.00	0.00
Enterobacter sp. strain BC dung	3	15.00	34.00	33.00	35.00
Enterobacter sp. strain MC dung	3	29.00	11.00	11.00	11.00
Brevibacillus sp. strain ELHPS3H13	3	23.83	15.00	15.00	17.00
Enterobacter sp. strain FU 103BA	3	25.17	16.00	16.00	17.00
Enterobacter sp. strain FU 104C	3	6.50	0.00	0.00	0.00
Paenibacillus sp. strain FU75CB	3	6.50	0.00	0.00	0.00
FU78CB	3	18.33	12.00	11.00	12.00
Control	3	11.57	11.70	11.00	12.00
$H(9)= 28.3805$, $P= 0.0008$					

There was significant ($p< 0.05$) difference of antibacterial activity of different isolates against *L. monocytogenes* $H\ (9) =28.3805$, $P= 0.0008$. *Enterobacter* sp. strain BC dung had significantly higher inhibition zone (*Md*=34mm) (Appendix IV e).

Table 14: Activity of the isolates on *K. pneumoniae*

Isolates	**N**	**Mean(mm)**	**Median(mm)**	**Minimum**	**Maximum**
BC5AC	3	10.33	10.00	9.00	12.00
B*revibacillus* sp. Strain BC5BC	3	8.00	0.00	0.00	0.00
Enterobacter sp. strain BC dung	3	29.00	30.00	30.00	30.00
Enterobacter sp. strain MC dung	3	8.00	0.00	0.00	0.00
Brevibacillus sp. strain ELHPS3H13	3	8.00	0.00	0.00	0.00
Enterobacter sp. strain FU 103BA	3	8.00	0.00	0.00	0.00
Enterobacter sp. strain FU 104C	3	8.00	0.00	0.00	0.00
Paenibacillus sp. strain FU75CB	3	26.00	15.00	15.00	15.00
FU78CB	3	21.50	12.00	11.00	13.00
Control	3	20.17	11.50	11.00	12.00
$H(9)=28.0$, $p= 0.0008$					

There was significant ($p< 0.05$) difference of antibacterial activity of different isolates against *K. pneumoniae* H (9) =28.374, P= 0.0008. *Enterobacter* sp. strain BC dung had significantly higher inhibition zone (*Md*=30mm) (Appendix IV f).

Figure 9: Antibacterial activity;

The plate shows the activity of isolates ELHP$_3$ H$_{13}$ (4), *Enterobacter* sp. strain FU 103BA (2), *Paenibacillus* sp. strain FU75CB (1), *Enterobacter* sp. strain FU 104C (3) and BC5AC (5) on *L. monocytogenes* with Ciprofloxacin (CIP) as a positive control (at the center).

CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Many members of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* are known to produce various cellulolytic enzymes i.e. endoglucanase, exoglucanase and β- glucosidases, (Orencio *et al*., 2016). The degradation of the Whatman No. 1 filter paper on Stan 21 agar was an indication of the activity of the cellulolytic enzymes. Species of genera *Enterobacter* are known to possess cellulolytic activity, for example, the isolates of *Enterobacter* strains isolated from the leaf litters in a botanical garden were found to possess cellulolytic activities (Akintola *et al*., 2021). The growth of the colonies on the surface and inside the media showed that the species of the genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* are facultative anaerobes. The colonies of genus *Brevibacillus* are smooth and light orange in color. The bacteriolytic activity of the isolates on dead *E. coli* cells in the water agar explained some antibacterial mechanism of the isolates on the test pathogenic bacteria. This was shown by the presence of clear zones along the streaks of the *E. coli* cells. The genus *Paenibacillus* has the ability to digest cells of other bacteria. Example, the strains of *Paenibacillus apiaries* formed clear zones on cells of *Arthrobacter citreus* SBUG 321 with lysis activity (Meene *et al*., 2022). The use of streptomycin solution in isolation media shows that the species of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* have developed antibacterial resistance. Bacteria in these genera possess extended spectrum β-lactamase, multiple drug resistance (MDR) and carbapeneum resistance (Singh *et al*., 2019). Stan 21 agar was the best isolation medium for genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* since it supported more growth than water agar. The presence of more bacteria growth in lake Bogoria indicated that it has favorable growth conditions compared to lakes Magadi and Elementaita. The difference in the bacteria growth among the three soda lakes can be

attributed by the differences in the salinity and alkalinity of different soda lakes (Banda, *et al*., 2020.

The morphological and biochemical tests showed the characteristics of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus*. Species of these genera are positive for nitrate reduction, sugar fermentation and negative for oxidase tests (De Oliveira *et al*., 2014; Thompson *et al*., 2018). The *Enterobacter* species which were majority (as seen in the BLAST search results) are Gram negative rods and this explains why most of the isolates were Gram negative rods. *Bacillus*, *Paenibacillus* and *Brevibacillus* species are Gram positive rods (Meena *et al*., 2014; Priest, 2015). The BLAST search placed the isolates into eight genera i.e. *Enterobacter*, *Brevibacillus*, *Paenibacillus*, *Providencia*, *Lysinibacillus*, *Bacillus*, *Pseudomonas* and *Escherichia*. The isolates had more than 95% sequence similarity to their closest relative in the BLAST, showing that they are likely to come from the same species as their closest relative. The identification of these genera whose members are found in the record of microorganisms already isolated in the Kenya's Rift valley, is a great achievement (Yadav *et al*., 2020; Sánchez *et al*., 2019).

The test pathogenic bacteria, *Salmonella spp*., *E. coli*, *K. pneumonia*e *Pseudomonas spp*., *S. aureus* and *L. monocytogenes* have been reported to gain antibiotic resistance for several human diseases (Church and McKillip, 2021). For example, resistance strain of *Salmonella* outbreaks was found to have a mortality rate of 3.4% compared to only 0.2 % of the sensitive strains (Brown *et al*., 2017). In the USA the annual health care cost of the resistant infections treatments was estimated to about US$ 4 billion (Murugaiyan *et al*., 2022). Methicillin-resistant *S. aureus* (MRSA) was first discovered in England in 1960s and since then it has become one of the leading cause of bacterial illnesses in health care (Lee *et al*., 2018). *E. coli*

and *K. pneumonia* uropathogenic strains have shown great resistance to beta-lactams, cotrimoxazole and quinolones (Ndzime *et al*., 2021). *L. monocytogenes* is known to have resistance to antibiotics like, ampicillin, gentamicin, erythromycin, trimethroprim, tetracycline and vacomycin (Baquero *et al*., 2020). Scientists are therefore discovering more antimicrobial compounds that can replace the already inactive antibiotics. The isolation of bacteria with antibacterial properties against these pathogenic bacteria in this study is therefore of notable contribution towards the discovery of new antimicrobial resistances.

Isolates *Enterobacter* sp. strain BC dung and *Enterobacter* sp. strain MC dung displayed broad- spectrum activity against the Gram negative and Gram-positive pathogenic bacteria. However, some Gram-negative bacteria, *Salmonella spp*. and *K. pneumonia* resisted the effects of *Enterobacter* sp. strain BC dung and *Enterobacter* sp. strain MC dung respectively. *Enterobacter* sp. strain BC dung had significantly higher antibacterial activity against *Pseudomonas spp*.(*Md*=16mm), *S. aureus* (*Md*= 35mm), *Listeria monoctogenes Md*= 34mm and *K. pneumoniae* (*Md* =30mm) (Tables 8, 11,12 and 13 respectively). *Enterobacter* sp. strain MC dung had higher inhibition zone *Md* = 25mm against *Salmonella spp.* (Table 9). *Enterobacter* sp. strain MC dung and *Enterobacter* sp. strain BC dung isolates were more effective on Gram positive than Gram-negative bacteria. For example, isolate BC dung did not have any antibacterial activity against *Salmonella spp.* Isolate *Enterobacter* sp. strain FU 103BA had a broad antibacterial activity against *P. aeruginosa* (*Md*= 12mm) and *L. monocytogenes* (*Md*= 16mm) (Tables 9 and 13). Isolate FU 104C had the least antibacterial activity among the *Enterobacter* isolates with a narrow antibacterial activity against only *P. aeruginosa* (*Md*= 15mm) (Table 9).

Studies have shown that different species of the genus *Enterobacter* produce secondary metabolites that possess broad-spectrum antibacterial activity (Biniarz *et al*.,2017). The observed antibacterial activities of *Enterobacte*r species from the crude extracts could be as a result of presence of active lipopeptides (Jemil *et al*., 2019). For example, various lipopeptides of *Enterobacter spp.* strain S-11 were found to possess broad biological activity against both Gram negative and Gram-positive bacteria. Oligopeptide produced by *E. cloacae* C3 strain expressed bioactivity against different pathogenic bacteria and fungi (Jemil *et al*., 2019). The most commonly known lipopeptide families include fengycin, surfactin, kurstakin and iturin (Fanaei *et al*., 2021). They were approved by the Food and Drug Administration (FDA) as antibiotics and have been in use in USA since 2003. Cubicin, a cyclic lipopeptide has been recommended by Food and Drug Administration for the treatment of skin diseases and blood infections caused by some Gram-positive bacteria (Hutchinson *et al*., 2017).

Isolates *Brevibacillus* sp. strain BC/5BC and *Brevibacillus* sp. strain $ELHPS_3H_{13}$ of genus *Brevibacillus* had significant ($P< 0.05$) antibacterial activity against *S. aureus* and *E coli* with *Md* =14mm, 25mm for *E. coli* and *Md*=11mm, 10mm for *S. aureus* respectively. In addition, *Brevibacillus* sp. strain $ELHPS_3H_{13}$ had antibacterial activity against *L. monocytogenes Md* = 15 (Tables 10, 11 and 12). Both isolates did not express any antibacterial activity against *Salmonella spp*., *K. pneumonia* and *Pseudomonas spp*. Studies have been conducted to demonstrate that *Brevibacillus* contain antibacterial substances like bacteriocin which could have contributed to the antibacterial activity of the isolates. For example, *Brevibacillus brevis* and *B. laterosporus* demonstrated to produce bacteriocin like inhibitory substances that is stable to changes in pH and temperatures (Omsap *et al*., 2016; Songnaka *et al*., 2021; Kumar *et al*., 2018). Bacteriocins are antimicrobial peptides that are grouped into different classes according to their structural and functional characterization (Chikindas *et al*., 2018).

Lantiobiotics which is a class I bacteriocin is the most studied and has wide applications in therapeutic and food industries. Unlike other secondary metabolite antibiotics, bacteriocins are ribosomally synthesized antibacterial peptides that kill related or non-related microorganism (Hernández *et al*., 2020). Bacteriocins that are produced by Gram negative bacteria include; colicins, nisins, pyocins, pediocins, bovocins and microcins. Colicins are antimicrobial proteins that kill related bacterial strains to the bacteria species that produce them to reduce the competition in their habitat. Gram-positive bacteria produce bacteriocins with low molecular weight and are grouped into three classes namely; lantibiotics, non-lanthiones and class III that are large protein and heat stable. *Brevibacillus brevis* is known to produce antibiotic peptide Gramicidin that is able to disrupt the lipid bilayer of microorganisms (Chen *et al*., 2020).

Paenibacillus sp. strain FU 75CB exhibited antibacterial activities against *Pseudomonas spp*., *Salmonella spp*., *E. coli*, and *K. pneumoniae*. There was no antibacterial activity against *S. aureus* and *L. monocytogenes*. This shows that the isolate FU 75 CB has narrow spectrum against only Gram-negative pathogenic bacteria. Various strains of genus *Paenibacillus* poses a great potential biological control agent through the production of different antimicrobial compounds. For example, *Paenibacillus elgii* (B69) produced the pelgipeptins A and B compounds that belong to family Polypeptin. Polypeptin is a peptide antibiotic that was first isolated from *Bacillus circulans* in 1948 and is active against many fungi and various Gram negative and Gram-positive bacteria (Kim *et al*., 2020). Twenty-five isolates of *Paenibacillus* from soil and water expressed broad-spectrum inhibitory effects against the test pathogenic fungi and bacteria. Fifteen strains of *P. polymyxa* produced inhibitory effects against *Phytophthora capsici* (Xu *et al*., 2016). Many soil *Paenibacillus* species are known to produce chitinases, cellusases, glucanase and proteases that are able to destroy eukaryotic cell

wall (Naing *et al*., 2014; Xie *et al*., 2016). Various isolates of *Paenibacillus* can therefore be used as a control of phytopathogenic microorganisms. For example, the enzyme extracted from *P. ehimensis* KWN38 expressed hyphal morphology deformation and prevented the development of phytopathogens such as, *Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici* (Naing, *et al*., 2015). *Paenibacillus* produce antimicrobial peptides with great potential application in medicine, food processing and agriculture industries (Jagadeesan *et al*., 2020). *Paenibacillus* antimicrobial peptides are ribosomally or non ribosomally synthesized. Ribosomally synthesized peptides include bacteriocins such as lantibiotics and pediocins while non-ribosomally synthesized include polymixin B, gramicidin A, alamethicin, gramicidin S (Yogeswaran *et al*., 2020; Krishnakumari *et al*., 2020).

P. polymyxa OSY-DF demonstrated antibacterial activities against various pathogenic bacteria. Various strains of *P. polymyxa* have demonstrated the production of polymyxin antibiotics which belong to a group of cyclic peptides having linear side chains (Huang and Yousef, 2015). Studies carried out on the *Paenibacillus spp* isolates, reported the discovery and characterization of lantibiotic (Park *et al*., 2017).

Paenibacillus have been known to produce various Exopolysaccharides (EPS) with application in the fields of medicine, cosmetics and bioremediation. *P. macerans* TKU029 produce EPS that possess natural skin moisturizer with potential application in cosmetics (Liang and Wang, 2015). Each strain of the genus *Paenibacillus* produce different types of exopolysaccharide. There are two types of commercially produced EXPs namely Levan- type

EPS that is produced by *P. polymyxa* EJS-3 and Curdlan gum produced by *P. polymyxa* ATTCC21830 (Liyaskina *et al*., 2021).

Isolate FU78CB belong to the genus *Providencia* in the BLAST search. The close relative species was *Providencia spp.* with 99.09% similarity. The isolate was found to have antibacterial activity against *Klebsiella pneumoniae* (*Md* = 12mm) and *L. monocytogenes* (*Md* = 12mm) (Tables 13 and 14). The results showed that the isolate has antibacterial activity against both Gram negative and Gram positive bacteria.

Isolate BC5AC was found to have antibacterial activity against *Klebsiella pneumonia* (*Md* = 10mm) (Table 14). According to BLAST search results, isolate BC5AC showed 99.51% similarity with *Lysinibacillus fusiformis*. Studies have shown that members of genus Lysinibacillus produce antimicrobial compounds. For example, *L. fusiformis* strain NBRC 15717 exhibited antifungal activity against *Aspergillus parasiticus* and *Aspergillus flava* both of which are aflatoxigenic (Khadka *et al*., 2020). This suggested that the newly isolated strain could be a potential candidate for production of antifungal compounds. In another study, *L. fusiformis* produced antifungal activity against *Rhizoctonia solani* (100% inhibitory effect) and *Fusarium oxysporum* (30% inhibitory effect) (Masri *et al*., 2021).

The results on antibacterial activities of the isolates discussed here shows that there are many undiscovered soil bacteria which are capable of producing various secondary metabolites with a wide application in pharmaceutical industries. Secondary metabolites include antibiotics (Perry *et al*., 2021), immunosuppressant (Baria *et al*., 2020), enzymes (Selim *et al*., 2021), siderophores (Kramer *et al*., 2020) and quorum- sensing molecules (Borges and Simões, 2019). Among the secondary metabolites, antibiotics have been for many years

isolated by the scientists for their uses in medicine most of them having been isolated from the members of *Actinobacteria*, *Firmicutes* and Myxobacteria (Mohammadipanah and Wink, 2016; Núñez and Barrientos, 2018; Braga *et al*., 2016). Members of the genera *Enterobacter*, *Paenibacillus*, *Brevibacillus*, *Lysinibacillus* and *Providencia* have shown that they have the ability to produce very useful antimicrobial metabolites against various pathogenic bacteria and fungi.

5.2. CONCLUSION

This study showed that the use of modified Stan 21 agar and water agar is ideal for isolation of 16 species of genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* in the soil samples from Lakes Magadi, Bogoria and Elementaita.

The 24 isolates obtained were examined for their biological activity against human pathogenic bacteria (*E. coli*, *Salmonella spp*., *K. pneumoniae*, *L. monocytogenes*, *S. aureus* and *Pseudomonas spp*.). The strains of species in the genera *Enterobacter*, *Paenibacillus* and *Brevibacillus* expressed antibacterial activities against the test pathogenic bacteria. The antimicrobial compounds extracted have potential uses in pharmaceutical industries.

5.3 RECOMMENDATIONS

The extensive screening of species of genus *Enterobacter*, *Paenibacillus* and *Brevibacillus* for their antimicrobial compounds is recommended. More experiments are needed to evaluate the bioactive substances from the crude extracts that are responsible for the biological activity in the isolated strains.

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APPENDICES

APPENDIX I: MEDIA PREPARATION

Stan 21 Agar

Ingredients

Solution A (700 ml of distilled water)

1g K_2HPO_4

0.02g Yeast extract

15g Agar

Solution B (300 ml of distilled water)

1 g KNO_3

1 g $MgSO_4$. $7H_2O$

1 g $CaCl_2$.2H2O

0.2 g $FeCl_3$

0.1 g $MnSO_4.7H_2O$

Preparation

Prepare solutions A and B separately by suspending 1g K_2HPO_4, 0.02g Yeast extract, 15g Agar, and 1 g KNO_3, 1 g $MgSO_4$. $7H_2O$, 1 g $CaCl_2$.2H2O, 0.2 g $FeCl_3$, 0.1 g $MnSO_4.7H_2O$ in 700ml and 300ml of distilled water respectively. Boil solution A to dissolve then autoclave both solutions separately at 121 ^oC at 15 lbs for 15 minutes before mixing them. Allow the medium to cool to 55° C then add 5ml of 10mg/ml of streptomycin. Pour the media into the Petri dishes. Place ($2cm^2$) sterile What man filter paper on the medium.

Water agar

Ingredients

1.5 g/L $CaCl_2$

1.5 g/L $MgSO_4$

11.9 g/L HEPES

15 g/L Agar

Preparation

Suspend 1.5 g $CaCl_2$, 1.5 g $MgSO_4$, 11.9 g HEPES and 15 g Agar in 1000ml of distilled water. Boil to dissolve then autoclave at 121 ^oC at 15 lbs for 15 minutes. Allow the medium to cool to 55° C then add 5ml of 10mg/ml of streptomycin. Pour the media into the Petri dishes. Using sterile swabs streak the dead *E coli* cells on the medium.

VY medium

Ingredients

5g/L Baker's yeast

1g/ L $CaCl_2.2H_2O$

15g /L Agar

Preparation

Suspend 5g Baker's yeast, 1g $CaCl_2.2H_2O$, 15g Agar in 1000ml of distilled water. Boil to dissolve then autoclave at 121 ^oC at 15 lbs for 15 minutes. Pour the medium into petri dishes.

CY medium

1g/L $CaCl_2$

1g/L Yeast extract

10g/L HEPES

16g/ L Agar

Preparation

Suspend 1g $CaCl_2$, 1g Yeast extract, 10g HEPES and 16g Agar in 1000ml of distilled water. Boil to dissolve then autoclave at 121 ^oC at 15 lbs for 15 minutes. Pour the medium into petri dishes.

Nitrate broth

Weigh 39 grams Nitrate broth and put in I liter distilled water. Heat the solution to dissolve the medium completely. Dispense the medium in test tubes and autoclave at 121°C and 15 lbs pressure for 15 minutes.

Triple Sugar Agar (TSI)

Dissolve 65 grams of medium in 1 liter of distilled water. Put the medium in test tubes and autoclave for 15 minutes at 121ºC and 15lbs pressure. Allow the medium to solidify in slope and a butt of 2.5 Inch long.

Sulphur- Indole Mortality (SIM) Agar

Formula

3.6% SIM

4% NaCl

1% Na_2NO_3

Preparation

Dissolve the components in 1000ml of distilled water. Boil with stirring the mixture to dissolve the medium completely. Dispense 3-10 ml of the medium in the test tubes and autoclave at 121°C and 15lbs pressure for 15 minutes. Allow the medium to make slants as it cools.

CY broth

1g/L $CaCl_2$

1g/L Yeast extract

10g/L HEPES

Preparation

Suspend 1g $CaCl_2$, 1g Yeast extract and 10g HEPES in 1000ml of distilled water. Boil to dissolve then autoclave at 121 °C at 15 lbs for 15 minutes. Pour the medium into petri dishes.

APPENDIX II: DNA EXTRACTION REAGENTS

DNA extraction buffer

Put 100ml of 1M EDTA (PH 8.0) in 500ml volumetric flask. Add 25 ml of 5 M NaCl and 50 ml Tris –cl solutions. Top with distilled water to 500ml mark.

10% SDS

Measure 5g of SDS. Put in 50ml tube then add 50ml of distilled water. don't autoclave. Always sore at room temperature.

5M NaCl

Dissolve 292.2g of NaCl in 1000ml of distilled water. Autoclave at 121°C for 15 minutes. Store the solution at room temperature.

CTAB/NaCl solution (2% CTAB and 0.7M NaCl)

Prepare 0.7M NaCl solution by dissolving 40.908g of NaCl in distilled water. Measure 2.0454 g of CTAB and top up with 50ml 0.7 M NaCl solution to make 50ml CTAB/ NaCl solution. Autoclave the solution and store at room temperature.

Chloroform: isoamyl alcohol (24:1)

Prepare the solution by mixing the components into the ratio of 24:1 respectively. CUOTION, work in the fume hood. Always store at room temperature and protect the solution from the light by wrapping the storage container using aluminum foils.

Isopropanol

Use absolute isopropanol. Always stored at -20° C.

70% Ethanol

Measure 70 ml of absolute alcohol (analytical grade) and top up to 100ml with distilled water.

Proteinase K

To one ml of ddH2O add 20 mg of Proteinase K (Promega # 52066) to make 20 mg /ml stock solution of proteinase K.

RNase A: Concentration of 10mg/ml

APPENDIX III: PCR REAGENTS

dNTPs

Working solution (100µl nucleotide mix) was prepared by adding 10 µl of d ATP, d CTP, d GTP, and d TTP each to 60 µl of water PCR grade.

Preparation of 16s Primers

Measure 90 µl of PCR water and top up with 10 µl of primer to make a concentration of 10 µM

Taq polymerase

PCR water

Buffer with $MgCl_2$

Genomic DNA Template

APPENDIX IV: ELECTROPHORESIS BUFFER WORKING CONCENTRATED STOCK

TAE Buffer 50x

Preparation

Weigh 242g of Tris Base and transfer to 2L conical flask. Add 700ml deionized water and mix until Trsi base dissolve completely.

Add 57.1 ml Glacial Acetic acid, 100ml of 0.5 M EDTA and Adjust PH to 8.3. Make the volume to 100ml using deionized water.

Sterilize the solution by autoclaving (20 minutes at 15lb/sq.in (psi) 121-124°C. Store the buffer at room temperature.

Preparation of 1x TAE Buffer

To prepare 1x TAE buffer solution, 1 volume of stock solution is mixed with 49 volumes of distilled water. For example, to prepare 100ml of 1x TAE solution from 100x stock solution measure 1 ml of stock solution and put in 100ml volumetric flask and top up with distilled water ($V_1C_1=V_2C_2$).

Gel Electrophoresis procedure

Measure (0.8% for genomic DNA and 1.2% for PCR DNA) of Agarose and put in 250ml conical flask. Add 100ml of 1X TAE buffer and heat to boil to dissolve completely. Allow the solution to cool. Add 1 µl of 10x Ethidium Bromide.

Place the bed on a level surface, add tooth comb and pour gently the solution into the bed. Let the gel solidify completely until it is firm to touch. Carefully remove the comb by slowly pulling it straight up. Place the gel into the electrophoresis tank. Carefully load the DNA together with the dye into the wells using 10 µl micropipette. Additionally, load 1 µl of the ladder with 2 µl into well no. 1. Run the gel at 100V for one and half hours. View the gel under UV trans illuminator.

EDTA 0.5 M pH 8.0

Dissolve 186.1 g of EDTA .2H_2O in 800 ml of distilled water. Stir the solution vigorously and simultaneously adjust the pH to 8.0 with Sodium Hydroxide pellets (EDTA will not dissolve until the pH is near 8.0). Bring the solution to a final volume of 1 liter using distilled water.

Ethidium Bromide l0×

Dissolve 1.0 g of EtBr in 100 ml distilled water. Cover the container with the aluminum foil and mix the solution by shaking. To make the 1× for gel staining, put 10 ml of the 10× stock in a 100ml volumetric flask and top up to 100 mark using distilled water. Store the EtBr solution at 4°C.

APPENDIX V: ANOVA TABLES

a) *Pseudomonas spp.*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Isolates	9	1382.483000	153.609222	153.2516	<.0001
Error	20	20.046667	1.002333		
Average scores were used for ties.					

b) *Salmonella spp.*

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Isolates	9	2142.332000	238.036889	11157.98	<.0001
Error	20	0.426667	0.021333		
Average scores were used for ties.					

c) *E. coli*

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Isolates	9	2274.800000	252.755556	64.2599	<.0001
Error	20	78.666667	3.933333		
Average scores were used for ties.					

d) *S. aureus*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Isolates	9	3905.361333	433.929037	128.4320	<.0001
Error	20	67.573333	3.378667		
Average scores were used for ties.					

e) *L. monocytogenes*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Isolates	9	3163.347000	351.483000	1077.067	<.0001
Error	20	6.526667	0.326333		
Average scores were used for ties.					

f) *K. pneumoniae*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Isolates	9	2659.675000	295.519444	824.7054	<.0001
Error	20	7.166667	0.358333		
Average scores were used for ties.					

g) Statistics for table of Source by bacteria growth

Fisher's Exact Test	
Table Probability (P)	<.0001
Pr <= P	<.0001

h) Statistics for table of media by bacteria growth

Fisher's Exact Test	
Cell (1,1) Frequency (F)	23
Left-sided Pr <= F	0.4192
Right-sided Pr >= F	0.7297
Table Probability (P)	0.1489
Two-sided Pr <= P	0.8384

APPENDIX VI: LIST OF SAMPLES

a. Samples from Lake Bogoria

LIST OF SAMPLES COLLECTED IN LAKE BOGORIA ON 17TH AND 18TH OCTOBER 2018.			
Site	Sample type	Purpose	GPS
Well 1(049)	Water x3 1.1,1.2,1.3	Isolation of Polyextremophil organisms	ELEVATION.3297feet. N..00°13.737'minute E. 036° 05.582' minute
	Soil x3(shore) 1.1,1.2,1.3	Isolation of Polyextremophil organisms	
Well 2 on the shore(050)	Water x3 2.1,2.2,2.3	Isolation of Polyextremophil organisms	ELEVATION.3298feet. N..00°13.690'minute E. 036° 05.606' minute
	Cowdung 1.1,1.2	Isolation of Polyextremophil	
	Soil x3 2.1,2.2,2.3	Comparison	
Well 3(052)	Water x1	Isolation for microorganisms	ELEVATION.3282feet. N..00°13.660'minute E. 036° 05.604' minute
	Soil 2.1	Comparison	
	Soil 3.1,3.2	Comparison	
	Algae x3 3.1,3.2,3.3	Isolation of enzymes for industrial application	

Sample site 4	Soil x3 4.1,4.2,4.3	Isolation of enzymes for industrial application	
Sample site 5	Soil x3 5.1,5.2,5.3	Isolation of enzymes for industrial application	
	Algae 5.1,5.2,5.3		
Sample site 6	Soil x3 6.1,6.2,6.3	Isolation of enzymes for industrial application	
Sample site 7	Feathers x 3 7.1,7.2,7.3	Isolation of enzymes for industrial application and comparison to other sites	
Sample site 8(gate entrance)(056)	Soil x2 8.1,8.2	Comparison	ELEVATION.3295feet. N..00°20.922'minute E. 036° 03.944' minute
	Scraping from salt rock	Composition	
	Dead flamingo on soil	Isolation of enzymes for industrial application	
	Insect dead flamingo	Comparison	
	Cowdung 8.3	Comparison	
	Soil(dead flamingo)	Isolation of enzymes for industrial application	
	Dead flamingo in water(063)	Comparison	ELEVATION.3262feet. N..00°20.969'minute E. 036° 03.898' minute
Sample site 9(057)	Cowdung x3 2.1,2.2,2.3	Isolation of enzymes for industrial application	ELEVATION.3294feet. N..00°20.930'minute E. 036° 03.832' minute
	Worm	Isolation of Polyextremophil	
	Tadpole	Isolation of Polyextremophil	
Sample site 9.1(060)	Water 1.1,1.2,1.3	Isolation for microorganisms	ELEVATION.3276feet. N..00° 20.932'minute

			E. 036° 03.835' minute
	Wood	Isolation of enzymes for industrial application	
	Insect	Isolation of enzymes for industrial application	
Sample site 9.2(058)	Cow dung 9.2	Isolation of enzymes for industrial application	ELEVATION.3280feet. N..00°20.912'minute E. 036° 03.828' minute
Sample site 9.3(059)	Cow dung x3 1.1,1.2,1.3	Comparison	ELEVATION...3276feet. N..00°20.916'minute E... 036° 03.826' minute
Sample site 9.4(061)	Water 1.1,1.2	Isolation for Polyextremophil	ELEVATION..3270feet. N..00°20.967'minute E.. 036° 03.867'minute
	Insect on water	Comparison	
	Tadpoles	Isolation of Polyextremophil	
	Soil x2 1.2,1.3	Isolation of enzymes for industrial application	
Inlet 9	Dead warm	Isolation of Polyextremophil	
	Dead insect	Isolation of Polyextremophil	
	Pieces of wood	Isolation of Polyextremophil	

b. Samples from Lake Magadi

Sampling Point	**Location & Elevation**	**Sample Type**	**Ph**	**Temp**	**Conductivity**	**Salt**	**TDS**
MP01	S02°00.069 E036°13.925 606M	Soil	9.51	67.5	433uS/cm	21.2ppt	28.6ppt
MP02	S02°00.037 E036°13.724 605M	Soil	10.3	55.0	21.4mS/cm	23.1ppt	27.6ppt
MP03	S02°00.024 E036°13.725	Soil	9.16	30.1	12.4mS/cm	20.1ppt	29.2ppt

	605M						
MP04	S01°59.975 E036°13.748 608M	Soil	10.4	27.8	20.3mS/cm	12.2ppt	23.7ppt
MP05	S01°59.925 E036°13.763 607M	Soil & sediment	9.20	23.4	46.8mS/cm	24.1ppt	21.9ppt
MP06	S02°00.091 E036°13.714 609M	Soil & sediment	8.97	25.7	34.1mS/cm	19.4ppt	19.0ppt
MP07	S02°00.144 E036°13.704 607M	Soil	10.60	28.9	23.7mS/cm	22.2ppt	29.3ppt
MP08S	S01°42.985 E036°16.277 618M	Water, Soil & sediment	9.65	30.8	30.5mS/cm	15.5ppt	20.3ppt
MP09S	S01°43.128 E036°16.263 618M	Water, Soil & sediment	9.37	28.6	46.4mS/cm	21.2ppt	30.2ppt
MP010S	S01°43.139 E036°16.265 616M	Water, Soil & sediment	9.10	38.7	40.5mS/cm	20.1ppt	26.1ppt
MP011S	S01°43.145 E036°16.266 615M	Water, Soil & sediment	8.90	66.8	43.0mS/cm	21.4ppt	28.4ppt
MP012S	S01°43.198 E036°16.253 614M	Water, Soil & sediment	8.88	74.3	45.3mS/cm	23.1ppt	29.5ppt
MP013S	S01°43.248 E036°16.235 617M	Water, Soil & sediment	9.16	32.4	36.3mS/cm	18.5ppt	24.4ppt
MP014S	S01°43.311 E036°16.228 612M	Water, Soil & sediment	9.18	77.8	46.5mS/cm	46.7ppt	30.8ppt
MP015S	S01°43.304 E036°16.279 613M	Water, Soil & sediment	8.70	69.1	43.6mS/cm	22.0ppt	29.1ppt
MP016S	S01°43.359 E036°16.284 615M	Water, Soil & sediment	9.23	28.6	49.6mS/cm	23.5ppt	31.2ppt